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RESEARCH PAPER

A rapid method for the recognition of the authenticity of edible oils and oil degradation based on TAG analysis using UHPLC-MS combined with chemometric tools

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Abstract

Authentication of edible oil is vital for health, economic, and commercial purposes. Separation and identification of triacylglycerols (TAGs) in edible oils can exploit genus-level differences in composition to recognise mixtures of oils of different plant origins. A novel direct method using ultra-performance liquid chromatography coupled to ion trap mass spectrometry was evaluated and validated for the discrimination of commercial edible oils based on TAG analysis. Statistical analysis of the dataset using principal component analysis (PCA) and cluster analysis was accomplished to study its distribution and determine the level to which oils from twelve different plant sources could be distinguished and authenticated. Sensitivity to post-production alteration was evaluated using a selection of aged and degraded oils. Using the PCA approach, some key TAG components such as trilinoleoylglycerol (LLL), 1-oleoylglycerol-2,3-dilinoleoyl (OLL*), 1,2-dioleoyl-3-linoleoylglycerol (OOL*), and trioleoylglycerol (OOO) were shown to be critical in detecting adulteration in high-value vegetable oils and estimating oil degradation under long-term storage and various heating conditions. This fast, simple, and reliable method offers significant benefits in authenticating edible oils and evaluating oil degradation. The method has excellent potential for universal use.

Keywords

Authentication, cluster analysis, degradation, edible oil, principal component analysis, triacylglycerols, ultra-performance liquid chromatography

Introduction

Triacylglycerols (TAGs) are the major form of dietary lipids in oils, as they generally represent 95–98% by mass (Lerma et al. 2011). TAGs are triesters of three fatty acids esterified to a glycerol backbone (Lichtenstein 2013). The compositions of the TAGs in vegetable oils exhibit differences at the genus level, suggesting that the composition of TAGs in oils differs depending on the source of the biological material (Barison et al. 2010; Tu et al. 2016; Chanidou 2020; Green et al. 2020). Moreover, it is suggested that the TAG profile is the best indicator of edible oil adulteration (Salghi et al. 2014). Recently, oil adulteration has become a focus of attention due to the food fraud issues involving the illegal mixing of expensive high-quality oils (e.g., olive oil and sesame oil) with cheaper oils (e.g., sunflower and rapeseed oil) with lower nutritional levels. Similarly, high-value extra virgin olive oil (EVOO) has been

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mixed with lower-value olive oil (e.g. refined olive oil) (Tu et al. 2016; Green et al. 2020). In particular, adulteration of high-value oils, including olive and sesame, esteemed for their taste and dietary benefits, has become an issue fuelled by both cost and demand (Xing et al. 2019).

Adulteration of oils has become a significant issue on a global scale (Vaclavik et al. 2011; Kou et al. 2018; Huq et al. 2022). According to the EU Agri-Food Fraud Network (FFN) and the Administrative Assistance and Cooperation System (AAC) (2019&2020), it is the most frequently reported product category in the system in 2019 and 2020 that relates to suspicions of fraud, with the majority of cases involving olive oil. Adulterated oils marketed as genuine oils cause economic damage to genuine oils producers and dealers, create commercial barriers between countries and adversely affect consumer rights (Marekov et al. 2010; Green et al. 2020). Therefore, it is important to verify that oils on the market are authentic not only to retain their high economic worth but also to ensure that consumers receive the high-quality products they have paid for (Green et al. 2020). In addition to commercial impacts, oil adulteration may make the products unsafe for human consumption. In extreme conditions, the consumption of adulterated oils has been linked to a wide range of diseases, including ulcers, cancer, asthma, and diarrhoea (Choudhary et al. 2020). "In the early 1980s, a syndrome referred to as the "toxic oil syndrome" occurred in Spain and resulted in massively reported illness and over three hundred deaths. The primary cause for the disease was the consumption of illegally marketed edible oil, adulterated with industrial-grade rapeseed oil containing 2% aniline as denaturant. This represents one of the most dramatic cases of health danger associated with adulteration" (Kamm et al. 2001).

Although edible oils play a significant role in human nutrition, degraded oils have potential health risks due to forming oxidised products that may have adverse biological activity (Bastos et al. 2017; Cao et al. 2019). Moreover, oil degradation could influence flavour stability and the quality of oil (Choe and Min 2007). Oils are subjected to auto-oxidation, which can become a significant process under various cooking conditions or during long-term storage (Choe and Min 2007; Zeb 2012). It has been claimed that the presence of polyunsaturated TAGs increases the potentially beneficial properties of the oils (Zarringhalami et al. 2011; Zeb 2012). On the other hand, polyunsaturated oils are more easily oxidised (Zeb 2012). According to published data, relatively few studies have investigated oil degradation (Zhang et al. 2012).

Various techniques have been developed to detect adulterants in edible oils and to study oil degradation. These include liquid chromatography-mass spectrometry (LC-MS) (Green et al. 2020), gas chromatography-mass spectrometry (GC-MS) (Xing et al. 2019), supercritical fluid chromatography-mass spectrometry (SFC-MS) (Tu et al. 2016), nuclear magnetic resonance spectroscopy (NMR) (Vigli et al. 2003; Fragaki et al. 2005; Tang et al. 2021), Raman spectroscopy (Zou et al. 2009; Zhao et al. 2022), and matrix-assisted laser desorption ionization-imaging mass spectrometry (MALDI-MS) (Cao et al. 2021). The direct detection of adulteration can be achieved using one of several fast techniques. It can be measured by NMR, Raman, or MALDI-MS, all of which characterise the oil as a "fingerprint" without performing any separation and pretreatment steps (Cao et al. 2021; Tang et al. 2021; Zhao et al. 2022). However, since there are no separation steps, these methods have low sensitivities to the detection of adulteration and, hence, can give inaccurate results (Tu et al. 2016). Moreover, some of these approaches are very expensive.

The detection of adulteration can be improved by applying a chromatographic approach combined with MS (such as HPLC-MS or GC-MS) since the efficient separation by chromatography and exact identification by mass spectrometry enables more comprehensive analysis, in the sense that it enables the determination of intact TAGs, allowing for the evaluation of potential variations in the TAG distributions (Tu et al. 2016; Wabaidur et al. 2016; Green et al. 2020). For the purpose of analysing TAGs, SFC-MS and GC-MS compete with HPLC-MS. The obvious advantage of the SFC approach is its short analysis time. However, it is noted that the resolution obtained by SFC is not as good as that using high-performance liquid chromatography (HPLC) (Rezanka et al. 2017; Salerno et al. 2023). GC-MS approaches can be more sensitive but are not direct and require pre-treatment steps such as a methanolysis step. Despite the fact that TAGs can be directly analysed using high-temperature gas chromatography-mass spectrometry (HTGC/MS), numerous studies have argued that some TAGs have been suspected to thermally degrade over 380 °C, particularly for unsaturated TAGs (Grob 1981; Byrdwell 2005; Sutton and Rowland 2012; Xu et al. 2018) since they are thermally unstable and can be subjected to autoxidation (Ikawa et al. 1997; Rezanka et al. 2017). Thus, the unsuitability of GC-MS analysis of TAGs paved the way for developing TAG analysis by liquid chromatography (LC) (Rezanka et al. 2017). HPLC-MS methods have now become the most widely used approaches due to essential advantages: a) their suitability for highly polar, high molecular mass, and thermally unstable compounds (e.g. polyunsaturated TAGs); and b) they are straightforward methods, in the sense that hydrolysis of analyte is not necessary as in GC analysis (Marikkar et al. 2005; Luccia et al. 2018). Although disadvantages still exist due to limitations in resolution, long separation times and detector selectivity, HPLC still offers considerable advantages as an analytical technique for the analysis of the TAG profiles of edible oils (Tsimidou et al. 1987). Numerous approaches have been developed to overcome these limitations, such as developing methods having shorter analysis time (Jakab et al. 2002; Lerma-García et al. 2011) and improving resolution (Ben Arfa et al. 2017; Green et al. 2020). However, none of the methods managed to achieve all the required objectives (e.g. speed and good resolution). Thus, establishing a method that combines and satisfies both elements is still of interest.

Recently, the UHPLC method developed by Chanidou (2020), coupled with ion trap mass spectrometry in the present study for the separation of TAGs, has the potential for use in authenticating edible oils and evaluating oil degradation under long-term storage and various cooking conditions. This approach to analysing intact TAGs has advantages over other published methods (Mottram et al. 1997; Jakab et al. 2002; Fauconnot et al. 2003; Holcapek et al. 2005; Lerma et al. 2011; Bosque-Sendra et al. 2012; Sutton and Rowland 2012; Tu et al. 2016; Ben Arfa et al. 2017; Green et al. 2020) in terms of better separation, speed, and simplicity. The key objective of the present study was to investigate the potential of the novel UHPLC method of Chanidou (2020) for the discrimination of various kinds of oils using chemometric tools (e.g. PCA and cluster analysis). Furthermore, the aim was to develop the application of the method for use as a tool to detect oil adulteration and degradation. To the best of our knowledge, it is the first time that the evaluation of oil degradation under long-term storage and various heating conditions was investigated on the basis of TAG analysis.

Materials and methods

Chemicals and reagents

Glyceryl trioleate (OOO; ≥ 98% purity) was purchased from Sigma-Aldrich (Merck KGaA , Darmstadt, Germany). HPLC grade solvents (hexane, dichloromethane, acetonitrile, 2-propanol, and methanol) and analytical reagent grade ammonium acetate were purchased from Fisher Scientific (Loughborough, UK).

Oil samples

A total of 152 vegetable oil samples from twelve types of oils (39 olive oil, 25 sunflower oil, 15 sesame oil, 13 rapeseed oil, 10 vegetable oil, 7 corn oil, 9 grapeseed oil, 8 walnut oil, 6 rice bran oil, 9 groundnut oil, 9 avocado oil, and 2 palm oil) were analysed for this study. Batch A (120 fresh samples) were collected in 2020–2022 from local stores in York, the UK, or Saudi Arabia or kindly donated by people. In addition, 14 mixed samples, 2 deep-fried samples, and 3 microwave samples were prepared from selected samples from batch A (Table 1). Batch B comprised 13 long-term storage samples (components had been stored in sealed glass bottles for approximately 5 years in the laboratory at room temperature).

Sample preparation

All vegetable oil samples were dissolved in hexane: acetonitrile: isopropanol mixture (1:1:1 *v/v/v*) prior to UHPLC analysis. In initial studies, different concentrations of the oil samples were prepared starting from a dilution of 100,000 times (dilution factor 10^5) to establish the appropriate concentration level. Following the examination of various concentrations of oil samples, all oil samples were diluted 1000 times (dilution factor 10^3).

Instrumentation

UHPLC method

For the separation of TAGs, an ultra-high-performance liquid chromatography (UHPLC) method was used, employing a Waters Acquity UPLC BEH C18 column (2.1 × 150 mm, 1.7 µm, 18% C loading) (Chanidou 2020). The column was maintained at 45 °C. The samples were dissolved in hexane: acetonitrile: isopropanol mixture (1:1:1 *v/v/v*), and the injection volume was 1 µL. The mobile phase consisted of A: acetonitrile, B: dichloromethane, and C: ammonium acetate in methanol (10 mM) (Table 2).

Table 1. Mixed and heated sample information. a) 100% pure sunflower and b) refined sunflower.

Sample type	Sample procedure
Mixed olive oil	Extra virgin olive mixed with 10% sunflower oil (in the laboratory)
Mixed olive oil	Extra virgin olive mixed with 20% sunflower oil (in the laboratory)
Mixed olive oil	Extra virgin olive mixed with 50% sunflower oil (in the laboratory)
Mixed olive oil	Extra virgin olive mixed with 15% rapeseed oil (in the laboratory)
Mixed olive oil	Extra virgin olive mixed with 30% rapeseed oil (in the laboratory)
Mixed olive oil	Extra virgin olive mixed with 50% rapeseed oil (in the laboratory)
Mixed olive oil	Extra virgin olive mixed with 15% rapeseed oil and 15% sunflower oil (in the laboratory)
Mixed sesame oil	Sesame mixed with 15% rapeseed oil (in the laboratory)
Mixed sesame oil	Sesame mixed with 30% rapeseed oil (in the laboratory)
Mixed sesame oil	Sesame mixed with 50% rapeseed oil (in the laboratory)
Mixed sesame oil	Sesame mixed with 15% sunflower oil (in the laboratory)
Mixed sesame oil	Sesame mixed with 30% sunflower oil (in the laboratory)
Mixed sunflower oil	Sunflower oil mixed with 15% extra virgin olive oil (by the producer)
Mixed palm oil	Palm oil mixed with 65% of sunflower (by the producer)
Deep-fried olive oil	Heated in an open pan on a hob for 10 mins (200 °C)
Microwave olive oil	Subjected to microwave heating for 5 mins (750–800W)
Deep-fried Sunflower oil	Heated in an open pan on a hob for 10 mins (200 °C)
Microwave sunflower oil a	Subjected to microwave heating for 5 mins (750–800W)
Microwave sunflower oil b	Subjected to microwave heating for 5 mins (750–800W)

Table 2. Mobile phase gradient of the method for UHPLC separation of TAGs. The mobile phase consisted of A: acetonitrile, B: dichloromethane and C: ammonium acetate in methanol (10 mM).

Time (min)	Flow rate (mL/min)	% A	% B	% C
0	0.55	85	10	5
15	0.55	70	25	5
18	0.55	65	30	5
20	0.55	60	35	5
21	0.55	85	10	5
26	0.55	85	10	5

Mass spectrometry (MS)

A Bruker HCT ultra-ETD II ion trap mass spectrometer with the APCI source operated in the positive ion mode under the following conditions: the vaporiser temperature = 450 °C; drying temperature = 300 °C; drying gas = 3 L/min; nebulizer gas = 45 psi; the capillary voltage = 15 V; the corona discharge voltage = 4000 V; the corona discharge current = 10 µA. Scan rate = 26000 m/z /sec. Scans were obtained over the range m/z 230–1100.

Statistical analysis

Statistical analysis was accomplished on the edible oil samples using past4.07 version software. PAST is a freeware data analyser app and calculator developed by Oyvind Hammer, University of Oslo, Norway (https://past.en.lo4d.com/windows). PCA and cluster analysis (Classical hierarchical) were performed on all oil samples analysed with the UHPLC-MS method. The database, which comprises sample names in columns and detected TAG mean relative peak areas in rows, was exported as Excel spreadsheets to perform PCA and classical hierarchical cluster analysis. The approach utilised the relative abundances of TAGs (RA%) since this is less variable than absolute peak areas of individual compounds, and the statistical processing requires data normalisation (variables were scaled to mean centred to reduce interferences). Specifically, the samples analysed in this study were the objects in the statistical analysis. The mean relative abundances of 62 TAGs were considered as variables in the data matrix for each oil sample (the different TAGs and their mean relative peak areas).

Results and discussion

UHPLC-MS method validation

As part of method validation, repeatability and reproducibility were assessed for key oils (olive, sesame, and sunflower). The relative standard deviation (RSD) of retention times for all selected TAG components ranged from 0.00 to 2.30% (Table 3), confirming high precision (all values < 5.00% as proposed by International Council for Harmonisation (ICH) guidelines (European Medicines Agency 2022) and by recent literature (Qian et al. 2020; Che Zain et al. 2021). For example, Che Zain et al. (2021) reported intra- and inter-day RSDs ranging from 0.04% to 1.74% for a UHPLC method, deeming the method precise since all RSDs were under 5%. The specificity was evaluated (following Food and Agriculture Organisation (FAO) guidelines) by inspecting chromatograms of blanks and standards, which revealed no extraneous peaks. Selectivity was verified by calculating the selectivity factor (α) for all major TAG components, which was > 1 (in the range 1.02–1.10). Although some α values were only slightly above 1 (e.g. 1.02), implying closely eluting peaks, they still signify that the peaks are resolved from one another. Common chromatographic criteria for adequate separation include a selectivity factor α > 1 and a resolution > 1.5–2 between adjacent peaks (Jehangir et al. 2017). In the current method, the α values > 1 confirm that no two major TAGs co-elute exactly, and visual examination of the chromatogram showed baseline or near-baseline separation for those critical pairs.

The TAG standard triolein (OOO) was chosen to assess the limit of detection (LOD) and limit of quantification (LOQ), as well as to determine linearity, since OOO is present in all samples. The LOD and LOQ were determined using the signal-to-noise ratio method recommended by the ICH guidelines, with the lowest concentration of the OOO standard (dilution factor 10^5). The chromatographic peak was above the LOD and LOQ (signal-to-noise ratio > 3 and > 10, respectively). The RSD values of the peak areas for OOO were calculated using three replicates at four concentrations (out of the six used for the calibration curve). The RSDs were 3.40, 5.00, 0.50, and 4.10% at dilutions of 1500, 2000, 2500, and 3000 times, respectively (Fig. 1). These values were within the acceptable range suggested by ICH guidelines. Another published method shows analogous precision: for instance, a high-temperature GC-FID method for TAGs achieved intraday retention time RSD < 0.5% and peak area RSD < 5%, with intraday variations in retention time < 1%. Although a larger day-to-day variance in peak areas (up to ~27%) was observed in that GC-FID study (likely due to complex matrix effects), the precision of the current method remained well-controlled (Qian et al. 2020). The calibration curve for OOO showed a linear response with $r^2 > 0.989$, indicating good linearity (Fig. 1). These findings are in agreement with other validated methods for TAGs and related lipids, which typically report high linearity (r^2 on the order of 0.99 or above). For instance, a GC-FID TAG analysis method showed regression coefficients from 0.990 up to 0.998 for various TAG standards across their calibration ranges (Qian et al. 2020). Likewise, an HPLC–MS method for vegetable oil TAGs obtained r^2 values exceeding 0.995 over a broad range (1–1000 µg/mL) (Ben Arfa et al. 2017). These examples underline that our r^2 ~0.99 is within the expected performance for a well-behaved analytical method. Overall, the novel UHPLC method proved to be robust, indicating its suitability for quantifying TAGs.

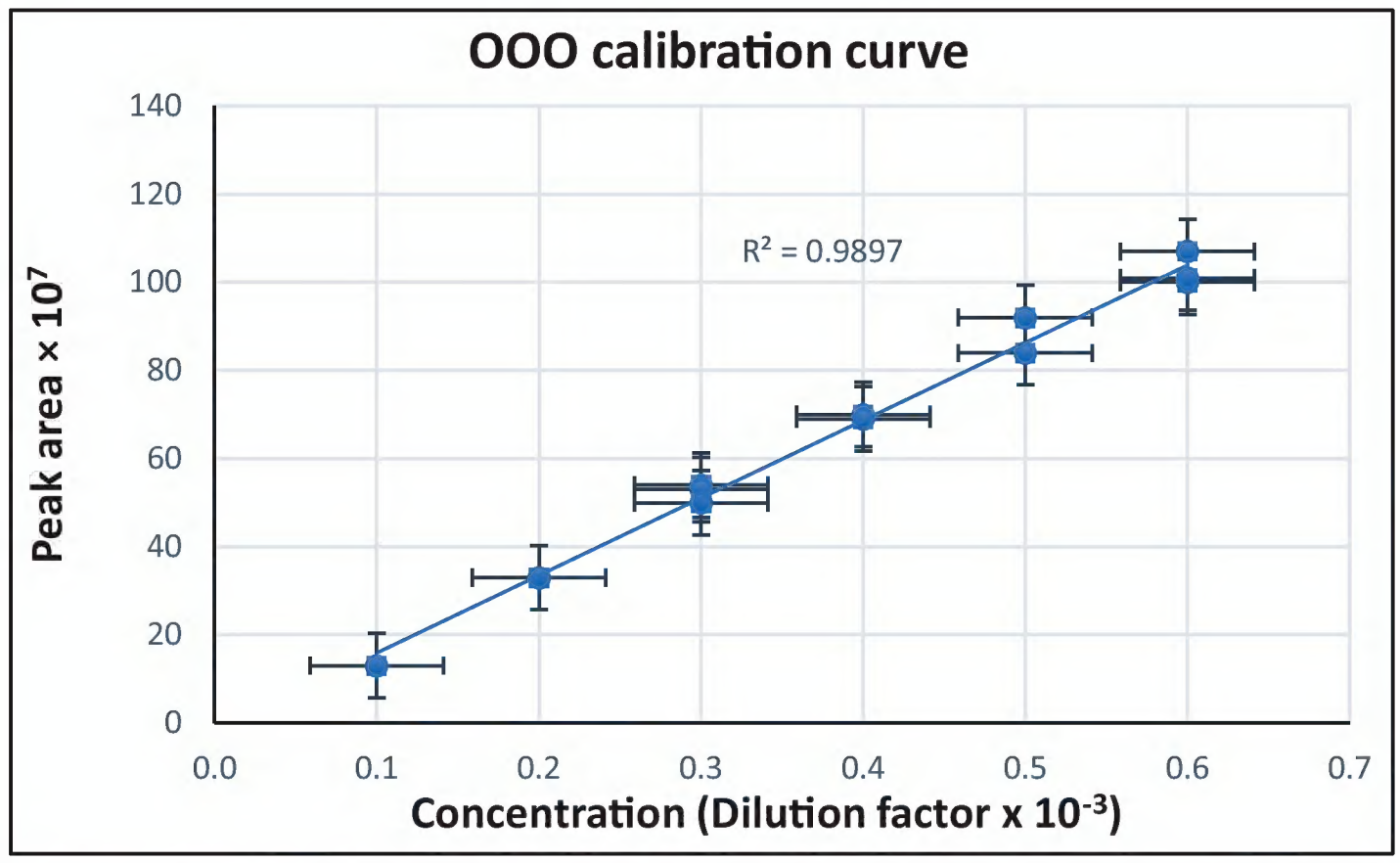

Figure 1. Calibration curve of the TAG standard OOO solution at six different concentrations (four concentrations among these concentrations were made up and analysed in triplicate to calculate the RSD).

Table 3. Standard deviation (SD) and relative standard deviation (RSD) of olive oil, sesame oil, and sunflower oil components for retention times and relative peak areas of selected TAGs from the start, the middle, and the end of the chromatogram.

Type of oil	TAG	Retention times			SD	RSD%	Relative peak areas			SD	RSD%
		RT (min) Exp.1	RT (min) Exp.2	RT (min) Exp.3			RA (%) Exp.1	RA (%) Exp.2	RA (%) Exp.3		
Olive (Intra-day)	LLL	7.20	7.30	7.50	0.15	2.00	0.50	0.46	0.49	0.02	4.10
	LOO*	10.20	10.40	10.60	0.20	1.90	31.78	31.32	31.07	0.36	1.10
	OOO	12.00	12.20	12.30	0.15	1.20	100.00	100.00	100.00	0.00	0.00
	OOP*	12.20	12.50	12.60	0.20	1.60	45.00	47.45	45.85	1.24	2.70
	OOS*	14.00	14.20	14.30	0.15	1.00	12.66	12.44	12.90	0.23	1.80
Sesame (Inter-day)	LLL	7.10	7.10	7.40	0.17	2.30	77.00	65.13	67.73	6.23	8.90
	LLO*	8.50	8.60	8.90	0.20	2.30	100.00	100.00	100.00	0.00	0.00
	LLP*	8.70	8.80	9.00	0.15	1.70	36.57	34.00	37.28	1.72	4.70
	OPS*	14.10	14.20	14.60	0.26	1.80	1.67	1.85	2.00	0.16	8.60
Sunflower (Intra-day)	LLL	7.00	7.00	7.00	0.00	0.00	100.00	99.80	100.00	0.11	0.10
	LLO*	8.30	8.40	8.30	0.05	0.60	99.94	100.00	95.80	2.40	2.40
	LLP*	8.50	8.60	8.50	0.05	0.60	36.99	35.73	34.78	1.10	3.10
	LOO*	10.00	10.10	10.00	0.05	0.60	54.90	52.70	52.23	1.42	2.60
	OOS*	13.60	13.80	13.70	0.10	0.70	3.22	3.59	3.48	0.19	5.50

Distribution of TAGs in edible oils

Twelve kinds of edible oil (olive, sunflower, sesame, rapeseed, vegetable, corn, grapeseed, walnut, rice bran, avocado, groundnut, and palm) were examined in this study using the UHPLC method (Chanidou 2020) coupled with ion trap MS. The individual TAG relative peak areas of all TAGs identified for edible oils were expressed relative to the total relative peak area (Table 4). TAGs were named using the conventional letter codes for the fatty acids (Persuric et al. 2018) and without identifying the *sn*-1/*sn*-3 positions on the glycerol backbone, since this is a limitation of the method. Thus, a TAG containing one oleic acid and two palmitic acids can be named OOP*, where the asterisk * indicates that the order in which the acyl groups are listed is not defined.

Among the oils examined, sunflower, grapeseed, walnut, and corn all contained LLL as the most abundant TAG (28, 36, 24, and 26%, respectively), whereas in olive oil, avocado oil, groundnut oil, and rapeseed oil, the most abundant TAG was OOO (47, 34, 44, and 29%, respectively) which was also abundant in vegetable oil (33%) (Table 4). Similarly, LLO/OLL is the dominant TAG in sesame oil (24%), while OOP/POO in palm oil and LOO/OOL in rice bran oil are the most abundant TAGs in such components (30 and 20%, respectively), as shown in Table 4.

In general, the triacylglycerol (TAG) profiles observed in this study are in strong agreement with previously reported data, confirming the robustness and reliability of the analytical method and its suitability for identifying and authenticating various edible oils. The predominant TAGs identified in sunflower oil (LLL 28%, LLO* 26%), corn oil (LLL 26%, LLO* 22%), and grapeseed oil (LLL 36%, LLO* 23%) closely match those reported by Jakab et al. (2002) and Holčapek et al. (2003), who found similar contents of these polyunsaturated TAGs, reflecting the high linoleic acid content typical of these oils. Similarly, the TAG profile of walnut oil was consistent with the results presented

Table 4. Mean relative abundance (%) of TAGs identified in different fresh vegetable oils (120 samples). These values are the average for each type of oil sample. The number of samples (*n*) and standard deviation (± SD) are given in the table. Carbon equivalent number (ECN) is defined as ECN = CN-2DB, where CN is the total number of carbons in all acyl chains, and DB is the number of double bonds in the TAG molecule.

ECN	TAGs	Corn *n* = 5	Groundnut *n* = 7	Grapeseed *n* = 8	Olive *n* = 27	Rapeseed *n* = 13	Rice bran *n* = 4	Sunflower *n* = 20	Sesame *n* = 9	Vegetable *n* = 10	Walnut *n* = 7	Avocado *n* = 9	Palm *n* = 1
38	LnLnLn										0.64 (± 0.04)		
	LLnLn*					0.41 (± 0.18)				0.20 (± 0.12)	4.83 (± 0.16)		
40	LLLn*	1 (± 0.35)		0.71 (± 0.63)		0.91 (± 0.31)			0.44 (± 0.60)	0.60 (± 0.40)	18.47 (± 0.24)		
	LnLnO*					2.47 (± 0.62)				1.64 (± 0.90)			
42	LLL	26 (± 1.67)	1.70 (± 0.61)	36.4 (± 1.28)	0.08 (± 0.16)	0.30 (± 0.44)	6.25 (± 0.23)	28 (± 1.56)	15.5 (± 1.60)	0.71 (± 1.70)	24.14 (± 0.89)	2.77 (± 0.22)	
	LnLO*				0.01 (± 0.04)	9.30 (± 1.41)				8.55 (± 2.24)	5.12 (± 0.72)		
	LnLP*					0.31 (± 0.44)			0.04 (± 0.12)		3.22 (± 0.22)		
44	LLO*	21.5 (± 1.41)	6.32 (± 0.43)	24.47 (± 1.50)	2.10 (± 0.86)	8.20 (± 0.42)	17.53 (± 0.44)	27.63 (± 1.24)	23.03 (± 0.47)	8.63 (± 1.46)	15.6 (± 0.84)	7.88 (± 1.25)	0.70
	LnOO*				0.34 (± 0.57)	12.4 (± 2.44)	11.67 (± 0.57)			9.12 (± 4.30)			
	LLP*	13.74 (± 1.08)	1.72 (± 0.42)	15.44 (± 1.16)	0.45 (± 0.51)			9.80 (± 1.09)	7.88 (± 1.34)		12.4 (± 1.31)	4.65 (± 0.47)	
ECN	TAGs	Corn *n* = 5	Groundnut *n* = 7	Grapeseed *n* = 8	Olive *n* = 27	Rapeseed *n* = 13	Rice bran *n* = 4	Sunflower *n* = 20	Sesame *n* = 9	Vegetable *n* = 10	Walnut *n* = 7	Avocado *n* = 9	Palm *n* = 1
44	LnOP*				0.15 (± 0.14)	0.42 (± 0.56)							2.56
46	GLL*												
	LOO*	14.26 (± 1.55)	13 (± 0.47)	5.50 (± 1.02)	14.1 (± 2.44)	23.82 (± 1.15)	20.75 (± 0.50)	15.15 (± 1.05)	21.32 (± 1.03)	27.1 (± 1.86)	5.50 (± 1.02)	10.96 (± 1.88)	2.45
	OOPo*											1.38 (± 1.52)	
	LLS*			5.29 (± 1.29)							2.46 (± 1.07)		
	OLP*	9.70 (± 1.47)	4.70 (± 0.33)	3.81 (± 1.35)	6.25 (± 1.47)	3.92 (± 0.99)	16.47 (± 0.60)	4.10 (± 0.55)	8.88 (± 1.03)	3.32 (± 0.60)	3.46 (± 0.84)	6.24 (± 0.68)	14.25
	OPPo*				0.01 (± 0.03)								
	LPP*	1.21 (± 0.72)	0.10 (± 0.21)		0.08 (± 0.15)		1.37 (± 0.40)	0.33 (± 0.33)	0.60 (± 0.27)				9.93
48	GLO*		0.92 (± 0.43)			0.14 (± 0.28)							
	OOO	7.55 (± 0.61)	42.71 (± 2.21)	3.21 (± 1.40)	47.4 (± 4.14)	29 (± 2.36)	16.75 (± 0.67)	10.4 (± 2.18)	13.17 (± 0.92)	33.1 (± 1.80)	2.90 (± 0.83)	34.77 (± 1.56)	5.23
	OLS*												
	OOP*	4.57 (± 0.51)	10.23 (± 0.96)	1.40 (± 0.83)	20 (± 1.50)	4.70 (± 1.10)	7.50 (± 0.41)	2.10 (± 0.56)	4.71 (± 0.83)	3.70 (± 1.44)	0.63 (± 0.25)	22.1 (± 0.98)	30
	OPP*	0.42 (± 0.25)	0.19 (± 0.24)		1.11 (± 0.43)				0.23 (± 0.19)			4 (± 0.65)	29.34
	PPP												
49	GOO*				0.12 (± 0.15)	1.20 (± 0.43)			0.02 (± 0.08)	1.61 (± 0.50)		0.28 (± 0.27)	
50	OLC_{20}		5.31 (± 0.30)						0.03 (± 0.05)				
	LLC_{22}							0.70 (± 0.10)					
	OOS*	0.70 (± 0.45)	3.80 (± 0.39)	0.99 (± 0.23)	5.60 (± 1.23)	1.80 (± 0.71)		1.22 (± 0.28)	2.89 (± 0.70)	0.77 (± 0.56)		3.20 (± 0.57)	2.10
	LSS*												
	POS*												
	OPS*				0.61 (± 0.52)			0.02 (± 0.03)	0.49 (± 0.25)			0.44 (± 0.51)	4.38
ECN	TAGs	Corn *n* = 5	Groundnut *n* = 7	Grapeseed *n* = 8	Olive *n* = 27	Rapeseed *n* = 5	Rice bran *n* = 4	Sunflower *n* = 20	Sesame *n* = 9	Vegetable *n* = 10	Walnut *n* = 7	Avocado *n* = 9	Palm *n* = 1
52	LLC_{24}							0.01 (± 0.03)					
	OLC_{22}		1.21 (± 0.17)					0.20 (± 0.20)					

ECN	TAGs	Corn *n* = 5	Groundnut *n* = 7	Grapeseed *n* = 8	Olive *n* = 27	Rapeseed *n* = 13	Rice bran *n* = 4	Sunflower *n* = 20	Sesame *n* = 9	Vegetable *n* = 10	Walnut *n* = 7	Avocado *n* = 9	Palm *n* = 1
52	OOC_{20}		1.72 (± 0.24)		0.32 (± 0.27)	0.50 (± 0.16)		0.01 (± 0.02)	0.12 (± 0.10)	0.56 (± 0.17)		0.22 (± 0.27)	
	OSS*				0.20 (± 0.63)				0.17 (± 0.15)				
54	OLC_{24}		0.27 (± 0.35)										
	OOC_{22}		3.94 (± 0.31)			0.10 (± 0.11)		0.07 (± 0.14)				0.30 (± 0.29)	
	OPC_{22}		0.19 (± 0.24)										
56	OOC_{24}		2.40 (± 0.24)										
	OPC_{24}		0.12 (± 0.15)										
58	OOC_{26}		0.21 (± 0.27)										

by La Nasa et al. (2013), with dominant levels of LLL and OLL, indicative of its polyunsaturated fatty acid-rich composition. In high-oleic oils such as olive, avocado, and groundnut, triolein (OOO) was the most abundant TAG, comprising 47%, 34%, and 44%, respectively, which aligns well with values reported by Jakab et al. (2002), further supporting the known fatty acid profiles of these oils and the analytical method's capacity for precise quantification. Rapeseed, sesame, and rice bran oils also exhibited TAG profiles in agreement with Endo et al. (2011) and Tu et al. (2016), with mixed oleic and linoleic acid TAGs (e.g., OOO, OOL, LLO) prevailing, reinforcing the expected composition of these oils. In the case of palm oil, POO* was identified as the major TAG, consistent with Fauconnot et al. (2003); however, Endo et al. (2011) reported OPP* (31%) and OOP* (24%) as more dominant, which may be attributed to differences in isomer quantification, instrument resolution, or sample source. Notably, this study is the first to report OOO (33%) and OOL* (27%) as the predominant TAGs in a specific oil, suggesting the presence of a high-oleic variant or a previously uncharacterized cultivar. This novel observation expands the existing knowledge of TAG distribution and highlights the need for continued investigation into cultivar-specific lipid compositions. Minor deviations across studies are considered acceptable and are likely the result of factors such as geographical origin, extraction or refining techniques, or cultivar differences. Overall, the close alignment of the current data with established literature confirms the accuracy and applicability of the method for profiling TAGs in a wide range of edible oils.

Consequently, the dominant TAGs described earlier can serve as markers for particular groups of oils, which can be further distinguished based on the presence of various less abundant TAGs. For example, the presence of OLnLn*, LnOL*, and OOLn* in rapeseed oil and vegetable oil components and the lack of these components in olive oil and groundnut oil further discriminates olive oil or groundnut oil from rapeseed oil or vegetable oil. This is explained by compositional differences: rapeseed oil typically contains α-linolenic acid (C18:3) at around 10% (double-zero varieties), whereas olive and groundnut oils contain virtually none. Thus, TAGs bearing linolenic acyl chains (Ln) — such as OLnLn, LnOL, and OOLn* — occur in rapeseed (and in generic "vegetable oil," which often is refined rapeseed oil) but are absent in olive or groundnut oil, aiding in distinguishing these oils. Overall, this research confirms previous findings and contributes to our understanding of the variations in TAG distributions of edible oils.

Principal component of different kinds of oils

In order to obtain a more precise visualisation of the difference between the samples and enable statistically based distinctions to be made, PCA and cluster analysis were applied. Distinction among the 12 different oils based on TAG profiles was demonstrated by PCA, the extent of variation between different oils being evident from their clustering in the scores plot (Figs 2, 3). PC1 to 3 contributed 92.12% of the variance in the dataset. The first and second principal components in the PCA scores plot show the distribution of various edible oils with 75.36% of variance from the first component (PC1) and 11.70% from the second component (PC2) (Fig. 2). The most obvious distinction among the oils, observed from PC1 vs 2 and PC1 vs 3 scores plot (Figs 2, 3), can classify the oils into three main groups. Group 1 encompassed oils abundant in mono-unsaturated TAGs (olive, avocado, groundnut, rapeseed, and vegetable), in which the combinations of OOLn*, OOL*, OOO, and OOP* (based on the loadings shown in Fig. 4) were the key contributors in isolating them from other oils. Group 2 comprised oils with high levels of di-/poly-unsaturated TAGs, including rice bran, sesame, sunflower, corn, walnut, and grapeseed. The combinations of LLLn*, LLL, OLLn*, LLO*, and LLP* (according to the loadings shown in Fig. 4) contributed significantly to setting these oils apart from the rest. Group 3 contained palm oil, which stood out as a saturated oil (discussed below).

In the PCA scores plot (PC1 vs 2), clear differentiation was achieved among ten kinds of edible oil (sesame, olive, corn/sunflower, walnut, grapeseed, rice bran, groundnut, avocado, palm, and rapeseed/vegetable), with rapeseed

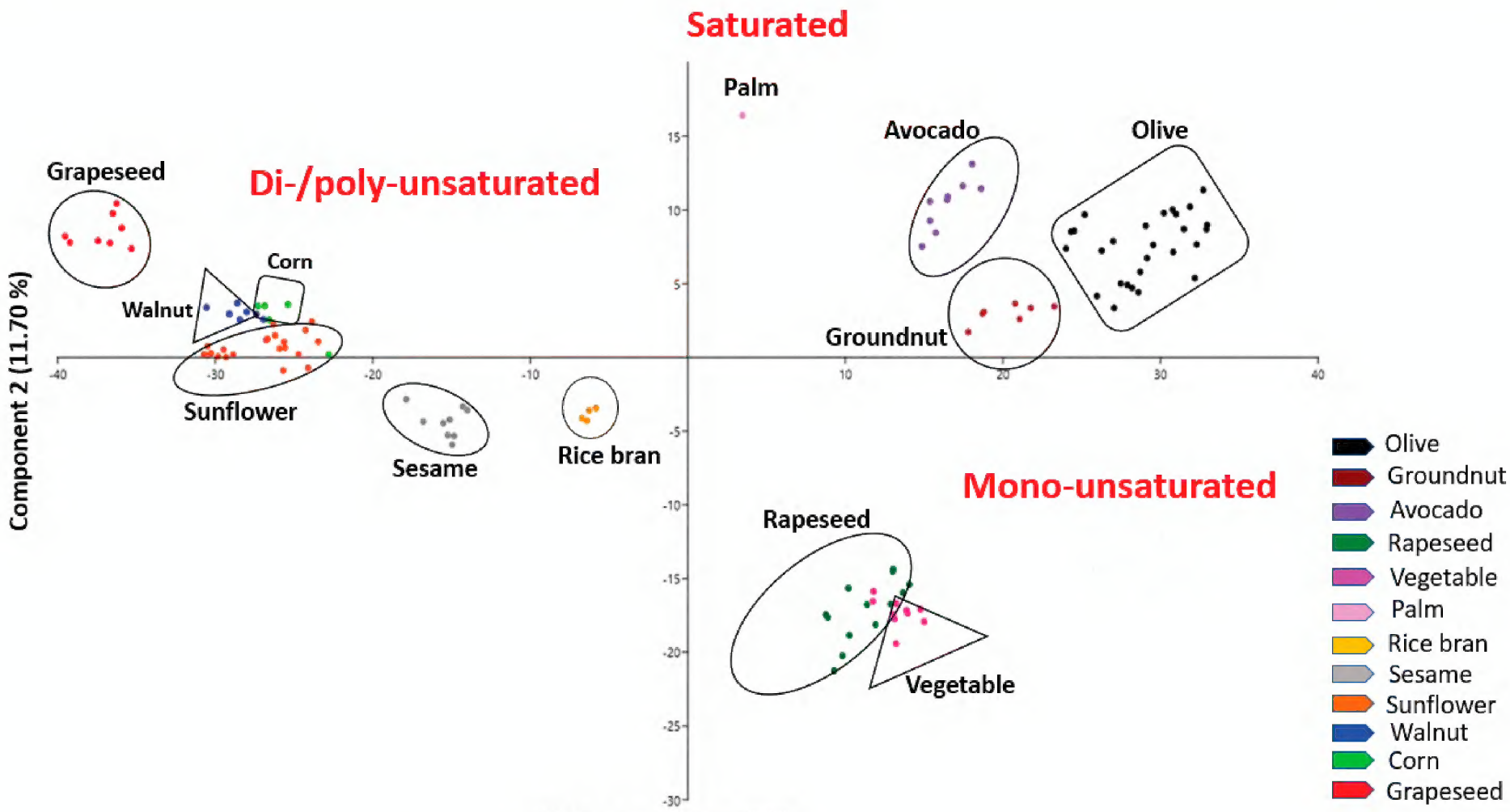

Figure 2. PCA scores plot (PC1 vs PC2) of 12 different kinds of oils, including olive oil, sunflower oil, sesame oil, rapeseed oil, vegetable oil, corn oil, grapeseed oil, walnut oil, rice bran oil, avocado oil, groundnut oil, and palm oil. All these samples were fresh.

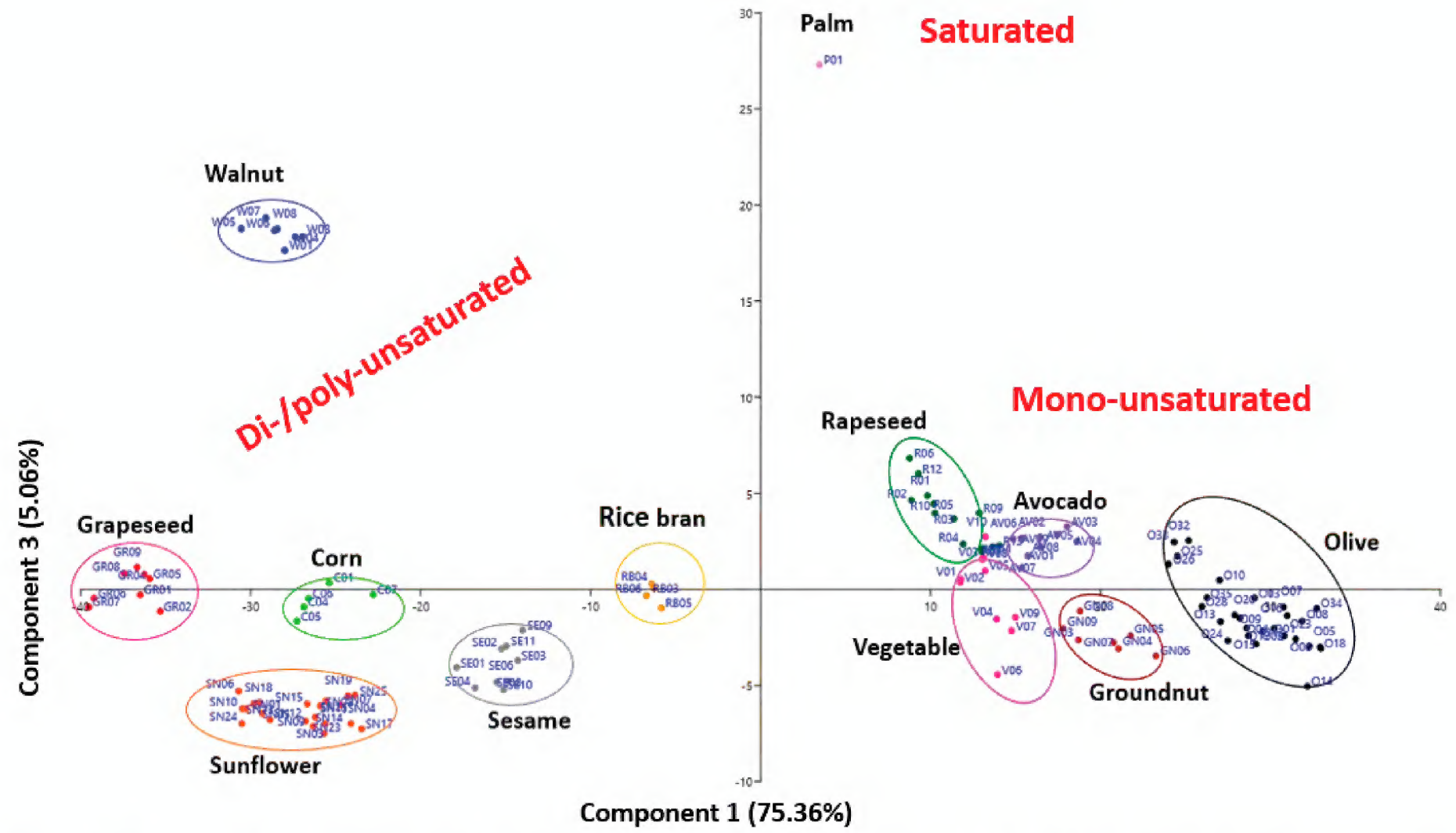

Figure 3. PCA scores plot (PC1 vs PC3) of 12 different kinds of oils, including olive (O), sunflower (SN), sesame (SE), rapeseed (R), vegetable (V), corn (C), grapeseed (GR), walnut (W), rice bran (RB), avocado (AV), groundnut (GN), and palm (P). All these samples were fresh.

and vegetable oils partly overlapping, as also observed between sunflower and corn oils (as shown in Fig. 2). However, in the PCA scores plot (PC1 vs 3), the twelve kinds of edible oil, including sesame, olive, corn, sunflower, walnut, grapeseed, rice bran, groundnut, avocado, palm, rapeseed, and vegetable, were discriminated well, and no overlap is observed (Fig. 3).

Based on the positive loadings from PCs 1 and 2 (Fig. 4), LLL, LLP*, OOO, OOP*, PPO* and OOS* were the most critical descriptors. Thus, they contributed significantly to the discrimination between olive oil, avocado oil, groundnut oil, palm oil, rapeseed oil, and vegetable oil in the PCA scores plot (PC1 vs 2), whilst LLL, LLO*, LLP*, OLLn*, LnOO*, and OOL*, having the highest negative loadings

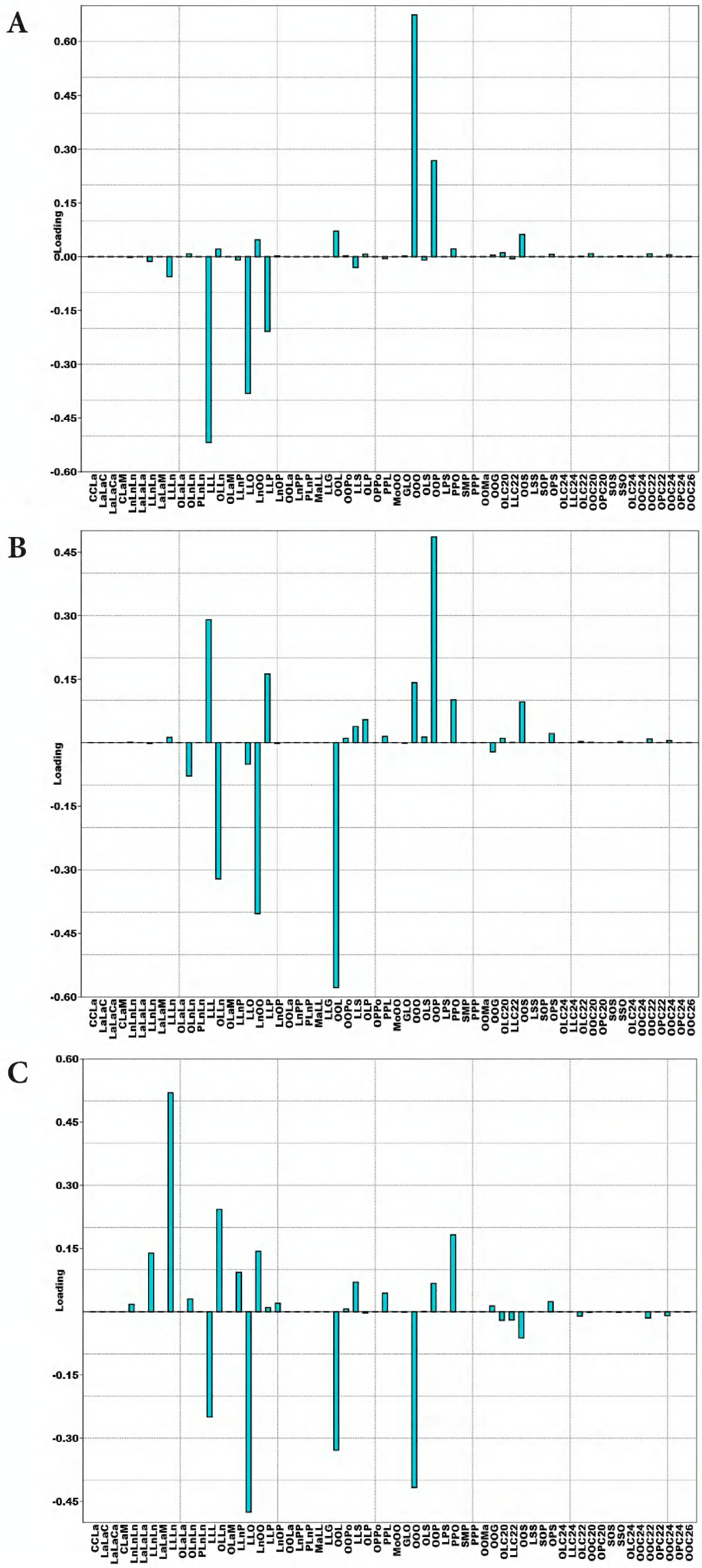
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Figure 4. A. PC1 loadings plot for Fig. 2; **B.** PC2 loadings plot for Fig. 2; **C.** PC3 loadings plot for Fig. 3.

(PC1 vs 2), contributed most to the discrimination between rice bran oil, sesame oil, sunflower oil, corn oil, walnut oil, and grapeseed oil.

Notably, the clusters of olive, avocado and groundnut occupy a similar area in the PCA scores plot since they have fairly similar major TAG abundances. However, based on the distributions shown in Table 4 and the loadings plot (Fig. 4), particular TAGs (OOO, OOP*, OPP*, OOPo*, OLC_{20}, OOC_{22}, and OOC_{24}) were important in discriminating olive, avocado, and groundnut from each other. The combination of OOP*, OLC_{20}, OOC_{22}, and OOC_{24} led groundnut oils to be separated clearly from olive and avocado oils, whereas OOO, OPP*, and OOPo* caused avocado oils to be isolated apart from olive and groundnut oils.

In the PC1 vs 2 scores plot, some vegetable oils fit with the variety of the examined rapeseed compositions, while others overlap. The range for vegetable oils is comparable to the ranges for a number of other oils, reflecting the variability that occurs naturally. The lack of a clear distinction between rapeseed and vegetable oil indicates a high degree of similarity in their TAG profiles. This is evident in the similar proportions of the TAGs LLL, LLO*, LLP*, LOO*, OOO, and OOS* (Table 4). Among the oils examined, vegetable oil is unique in that it contains a blend of different compositions. Typically, vegetable oil (it is a general description used for mixed oils) reflects a high rapeseed oil content mixed with a low percentage of another oil (e.g. sunflower, palm, safflower, and soybean). Both rapeseed and typical vegetable oil blends are rich in monounsaturated oleic acid and polyunsaturated linoleic acid, with comparable levels of polyunsaturates like linoleic (C18:2) and even linolenic (C18:3) in many cases. For example, a canola-based vegetable oil might contain ~20% linoleic and ~10% α-linolenic acid – similar to pure canola – whereas a soybean-based vegetable oil has ~50% linoleic and ~7% linolenic. These similarities in fatty acid composition translate to overlapping TAG profiles. Studies have shown that rapeseed and soybean oils tend to cluster together in multivariate analyses due to their chemical resemblance (Wu et al. 2019). However, the distinction between rapeseed and vegetable oils is observed most clearly using the PCA scores plot (PC1 vs 3). Sunflower oil, corn oil and walnut oil were very well separated in PC3, as shown in Fig. 3. LLLn*, LnLO*, PPO*, having the highest positive loadings, LLO*, OOO, and OOL* (the highest negative loadings) were the most essential descriptors in PC3, contributing significantly to the discrimination on PC3.

Specifically, in PC3, LnOO* and OOL* contributed significantly to the discrimination between rapeseed and vegetable oils, whereas LLO* and OOO contributed to the distinction of corn from sunflower. This matches prior chemometric analyses that differentiate high-linoleic vs higher-oleic oils by their TAG patterns (Chanidou 2020). Sunflower oil (traditional linoleic type) is well known to be extremely high in linoleic acid and low in oleic acid, whereas corn oil, while also linoleic-rich, contains slightly more oleic acid. As a result, sunflower oil's TAG profile is dominated by di-linoleoyl TAGs (like LLL and LLO), whereas corn oil has a greater relative abundance of oleic-containing TAGs (like OOL and OOO) (Chanidou 2020).Interestingly, both the distributions (Table 4) and the positive loadings plot for PC3 reveal that a combination of LLnLn*, LLLn* and LnLO* caused walnut oils to be significantly separated from sunflower, corn and grapeseed oils on PC3. Walnut oil formed a clearly isolated cluster on PC3, which we attribute to its unique TAG composition, particularly the presence of TAGs with multiple linolenic or combined linoleic–linolenic acyl chains (e.g., LLnLn*, LLLn*, LnLO*). Walnut oil is notable for its content of α-linolenic acid (~8–13%) alongside a high linoleic acid (~60%) (Bouabdallah et al. 2014). Similarly, the proportion of OOP* and PPO* for palm oil, being significantly higher than for other oils, and the positive loadings plot for PC3 exhibits that the combination of OOP* and PPO*, having the highest magnitude, caused the palm component to be significantly isolated from other oil components on PC3. This can be understood from the fatty acid composition: palm oil contains ~45% palmitic (C16:0) and ~40% oleic (C18:1) acids, leading to correspondingly high levels of palmitic–oleic TAG species (PPO, OOP). These TAGs are far more abundant in palm oil than in oils with lower saturated fat content, explaining palm oil's distinct separation.

The proportion of LLL for grapeseed being higher than for other polyunsaturated oils caused such oil to be discriminated well from sunflower, corn and walnut on PC2. In addition, LLS* was only detected in grapeseed and walnut, and thus this causes those samples to separate further from sunflower oil and corn oil on PC2 and 3. The loadings plot for PC3 alongside the distributions reveals that a combination of OOL* and LLS* caused grapeseed and walnut to be clearly distinguished from corn and sunflower on PC3.

Until now, there has been limited literature concerning classifying edible oils based on TAG analysis using HPLC through the application of PCA (Lisa et al. 2009; Green et al. 2020). Green et al. (2020) succeeded in distinguishing only five kinds of edible oil with 82% of the total variance from PC1 and PC2 in a relatively short analysis time. On the other hand, Lisa et al. 2009 managed to distinguish a wide range of edible oils, but this required a much longer separation time, exceeding 100 min. In addition, a comparison of the level of distinction between the different oil clusters in this approach versus Lisa et al. (2009) reveals that the groupings in the present study showed more distinct separation through PCA in a single analysis. In contrast, Lisa et al. (2009) reported merely one sample (n = 1) for the majority of the oils analysed (e.g. corn, sesame, walnut, and avocado). This limited sample size is insufficient to categorise such oils effectively into distinct groups. To the best of our knowledge, this study represents the first

instance of successfully classifying 12 types of edible oil in a short analysis time using such an approach. An important issue emerging from these findings is that this approach has great potential in detecting oil adulteration, especially in high-value oils.

Cluster analysis of different kinds of oils

Cluster analysis was used alongside PCA in the present study to gain a comprehensive understanding of the relationships in TAG profiles within various kinds of edible oils. However, the difference between these two approaches in this regard is that PCA aims to reduce dimensionality and identify the significant combinations of TAGs that contribute to overall variance, whereas cluster analysis groups oils based on their similar TAG distributions (Kalogiouri et al. 2021). Cluster analysis is used to divide a collection of objects into various clusters by measuring the statistical distance between each pair of objects in terms of variables and combining those that are close together (Kalogiouri et al. 2021). Various distance metrics are available for measuring the distance between variables, such as Euclidean distance, Cosine distance, Manhattan distance, and Bray-Curtis distance. Among these options, the Euclidean distance approach is one of the most commonly employed for measuring the distance between data points in multi-dimensional space since it is straightforward to interpret and simple to compute (Zhou et al. 2017; Granato et al. 2018). In this study, a dendrogram was created using hierarchical cluster analysis (HCA) based on Euclidean distance and the paired group algorithm to identify the oil groups with objects that have a high degree of similarity. Each object (oil sample) is treated as a singleton cluster by the algorithm. Then, pairs of clusters are merged until a large cluster containing all of the objects is formed, resulting in a dendrogram (Kalogiouri et al. 2021).

As shown in Fig. 5, twelve oil clusters were identified with different distances among the various oils from one cluster to another, which indicates that they were successfully distinguished. In this investigation, three major groups were observed according to the saturation type of oils (Fig. 5). Group 1 clustered oils with high contents of di-/poly-unsaturated TAGs, including rice bran, sesame, sunflower, corn, walnut, and grapeseed. Interestingly, the walnut oil cluster is relatively far from the rest of the polyunsaturated oils, consistent with the previous result shown in Fig. 3. Group 2 encompassed samples rich in mono-unsaturated TAGs (olive oil, avocado oil, groundnut oil, rapeseed oil, and vegetable oil), among which rapeseed and vegetable oils exhibited a slight distance from olive, avocado, and groundnut oils. Lastly, group 3 contained palm oil, characterised by its abundance of saturated FAs.

The olive cluster exhibited several small subgroups, indicating potential variations among the sources of

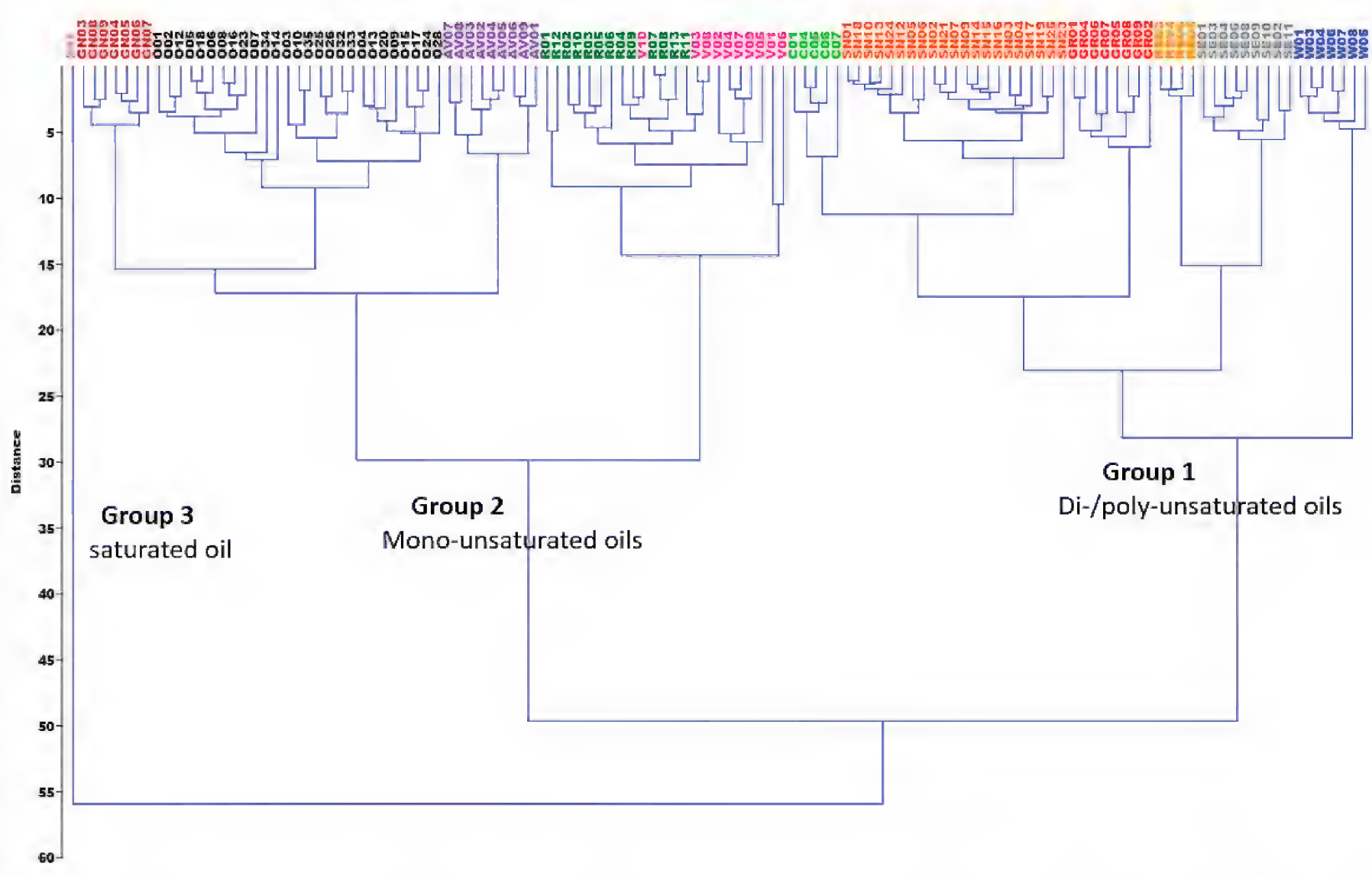

Figure 5. Hierarchical cluster analysis by linkage and Euclidean distance obtained from 12 different kinds of oils, including olive oil (O) (black), sunflower oil (SN) (orange), sesame oil (SE) (grey), rapeseed oil (R) (dark green), vegetable oil (V) (pink), corn oil (C) (bright green), grapeseed oil (GR) (red), walnut oil (W) (blue), rice bran oil (RB) (gold orange), avocado oil (AV) (violet), groundnut oil (GN) (brown), and palm oil (P) (purple). All these samples were fresh.

olive oil. Notably, the vegetable cluster was most similar to the rapeseed group due to their compositional similarities in their TAG abundances. However, the discrimination between rapeseed and vegetable groups was observed much more clearly in the cluster analysis shown in Fig. 5 than by PCA (Fig. 2). Notably, vegetable oil (V10) clusters tightly with rapeseed oils, indicating that it could be 100% rapeseed oil that is labelled as vegetable oil (this information was confirmed after referring to the label of the oil package). This finding highlights how chemometric profiling can uncover mislabelling. "Vegetable oil" is a generic label often applied to inexpensive refined oils (commonly rapeseed or soybean). In this case, the analysis correctly identified the so-called vegetable oil as actually being rapeseed oil, underscoring the utility of this method for verifying label claims. Overall, these results reinforce the conclusions drawn from the previous PCA findings and contribute to the classification of various types of edible oil.

Detection of oil adulteration

In order to establish a method for the identification of adulterants in high-value oils according to their TAG profiles, several model samples of olive oil and sesame oil were artificially adulterated by the addition of different proportions of cheaper oils, such as sunflower and rapeseed (details of samples reported in Table 1).

The chromatograms of mixtures of olive, sesame, and sunflower oils at the various mixture levels are shown in Figs 6 (olive with sunflower), 7 (sunflower with olive), and 8 (sesame with rapeseed). As the sunflower oil concentration in the olive oil sample increased, the relative abundances of the TAG markers of sunflower LLL and LLO* increased gradually (e.g. relative abundance of LLL was increased from 1% to 78% at various mixture levels) (Fig. 6). Fig. 7 shows the TAG marker of olive oil (OOO), which increased in the sunflower oil sample when adding 15% of olive oil compared to pure sunflower oil. In addition to that, the proportion of OOS/SOO, which is more abundant in olive oil than in sunflower oil, increased from 1% to 7%. As shown in Fig. 8, when gradually increasing the concentrations of rapeseed oil in the sesame oil sample (15%, 30%, and 50%), the relative abundance of the TAG marker of rapeseed oil (OOO) was increased (from 36% to 80%), and the proportion of the abundant TAG (LLL) in sesame oil was decreased (from 74% to 52%). Moreover, certain triacylglycerols (TAGs), specifically LLLn*, LnLnO*, and LnLO*, which were not detected in pure sesame oil, were clearly present in the adulterated (mixed) samples. These TAGs are characteristic of oils containing appreciable amounts of linolenic acid, such as rapeseed oil. This observation is supported by the chromatographic profile of the sesame–rapeseed blend, where these TAGs are distinctly observed in the mixed sample but are absent in the chromatogram of the pure sesame oil (Fig. 8), thereby

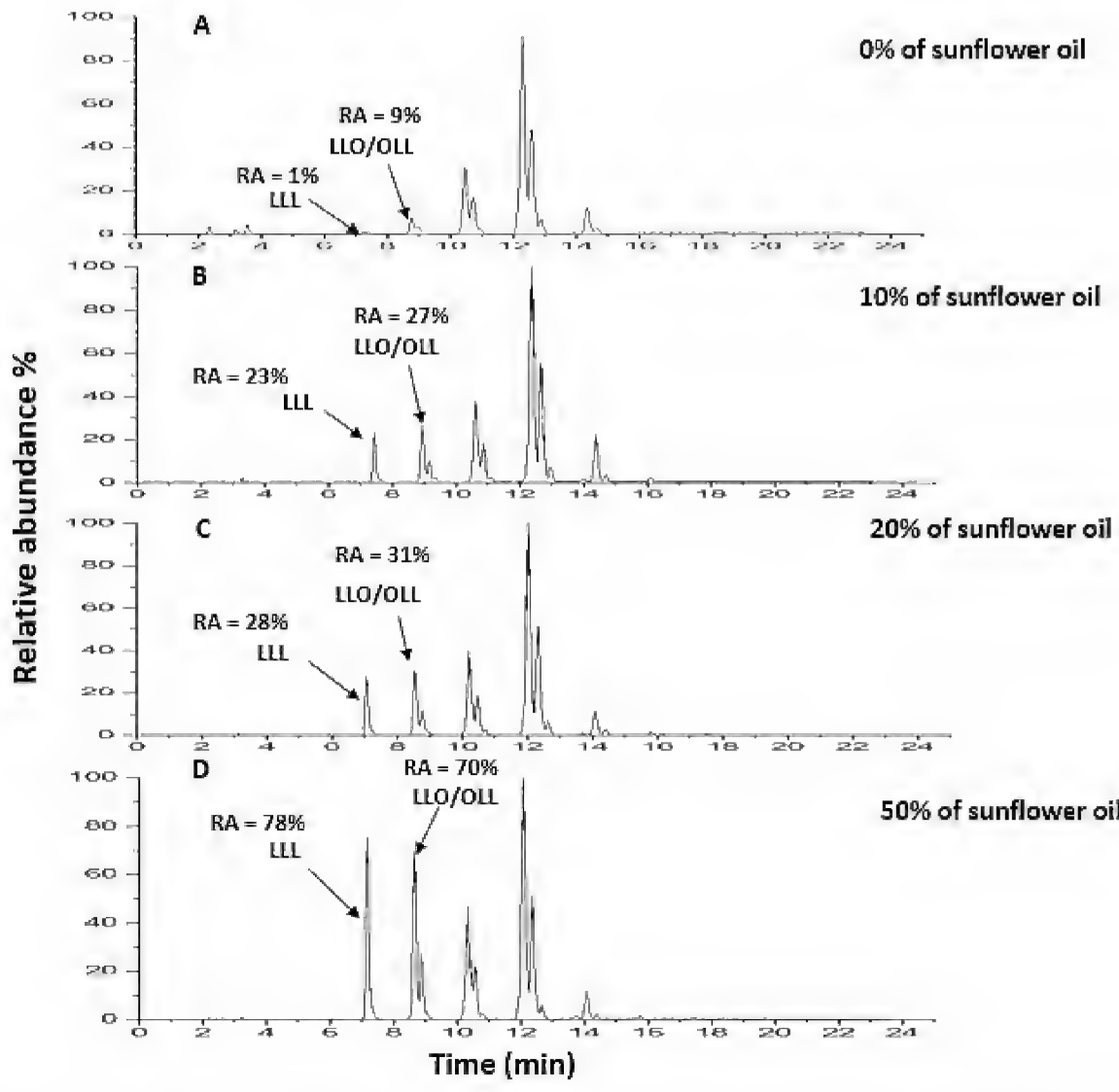

Figure 6. UHPLC-MS chromatograms of **A.** Pure olive oil; **B.** Olive oil mixed with 10% of sunflower oil; **C.** Olive oil mixed with 20% of sunflower oil, and **D.** Olive oil mixed with 50% of sunflower oil, showing the TAG marker of LLL and LLO*. These samples were mixed in the laboratory.

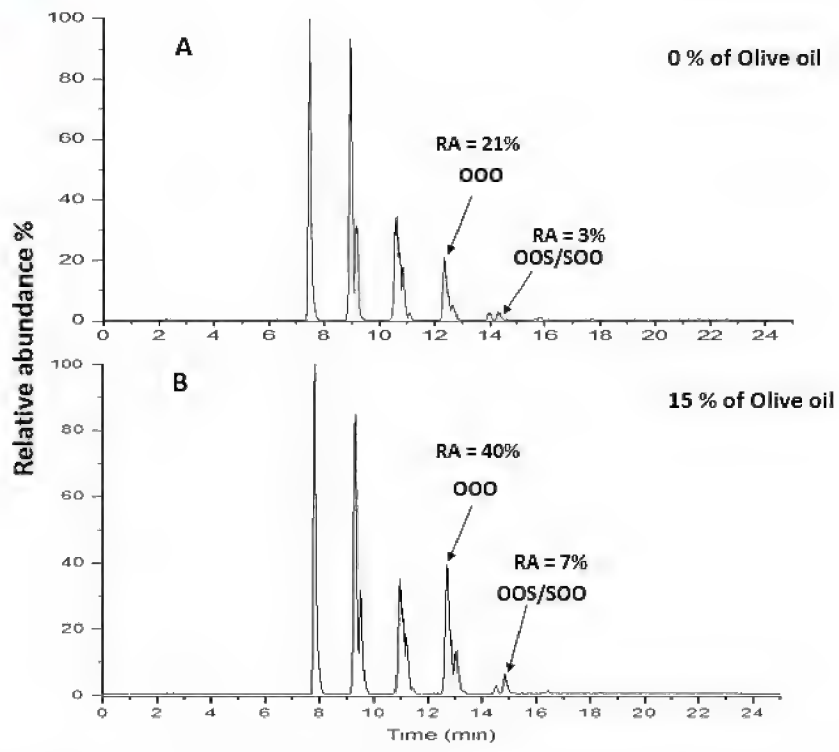

Figure 7. UHPLC-MS chromatograms of **A.** Pure sunflower oil, and **B.** Sunflower mixed with 15% of olive oil (this sample was mixed by the producer), showing the TAG marker of OOO.

confirming their role as reliable markers of adulteration. The presence of such TAGs serves as a strong indicator of non-authenticity, as genuine sesame oil is typically devoid of linolenic acid and its derived TAG species. Similarly, the TAG profile of olive oil, characterised by a high abundance of OOO and low levels of LLL, could serve as a reliable indicator of authenticity. In contrast, sunflower oil contains significantly higher concentrations of LLL and lower levels of OOO. As a result, the adulteration of olive oil with sunflower oil leads to a discernible increase in LLL and a corresponding decrease in OOO. Although these compositional changes may be subtle, they are consistent and quantifiable, making them effective markers for detecting adulteration.

When subjected to multivariate statistical analysis, such as PCA, variations in TAG composition (specifically, the appearance of linolenic acid-containing TAGs in adulterated sesame oil and shifts in the OOO/LLL ratio in olive oil) produce clear separation between authentic and adulterated samples. These compositional differences manifest as distinct clustering patterns in PCA score plots, thereby enhancing the accuracy and reliability of adulteration detection.

To further examine the robustness of the approach in detecting adulteration involving mixed oils with quite similar major TAG abundances, olive oil was mixed with various concentrations of rapeseed (15%, 30%, and 50%), which both have comparable major TAG profiles (e.g. OOO and OOL*). Additionally, sesame oil was mixed with two concentrations of sunflower oil (15% and 30%). Then, the data for these mixtures were subjected to PCA and HCA analysis. Adulteration between oils with similar TAG compositions (e.g., high-oleic oils like olive and rapeseed) is particularly challenging to detect. Including these difficult cases allowed us to rigorously evaluate the method's detection capability.

In order to analyse the underlying patterns and the broader compositional changes of mixtures, PCA was utilised. Fig. 9 shows the scores plot of PC1 and 2, which

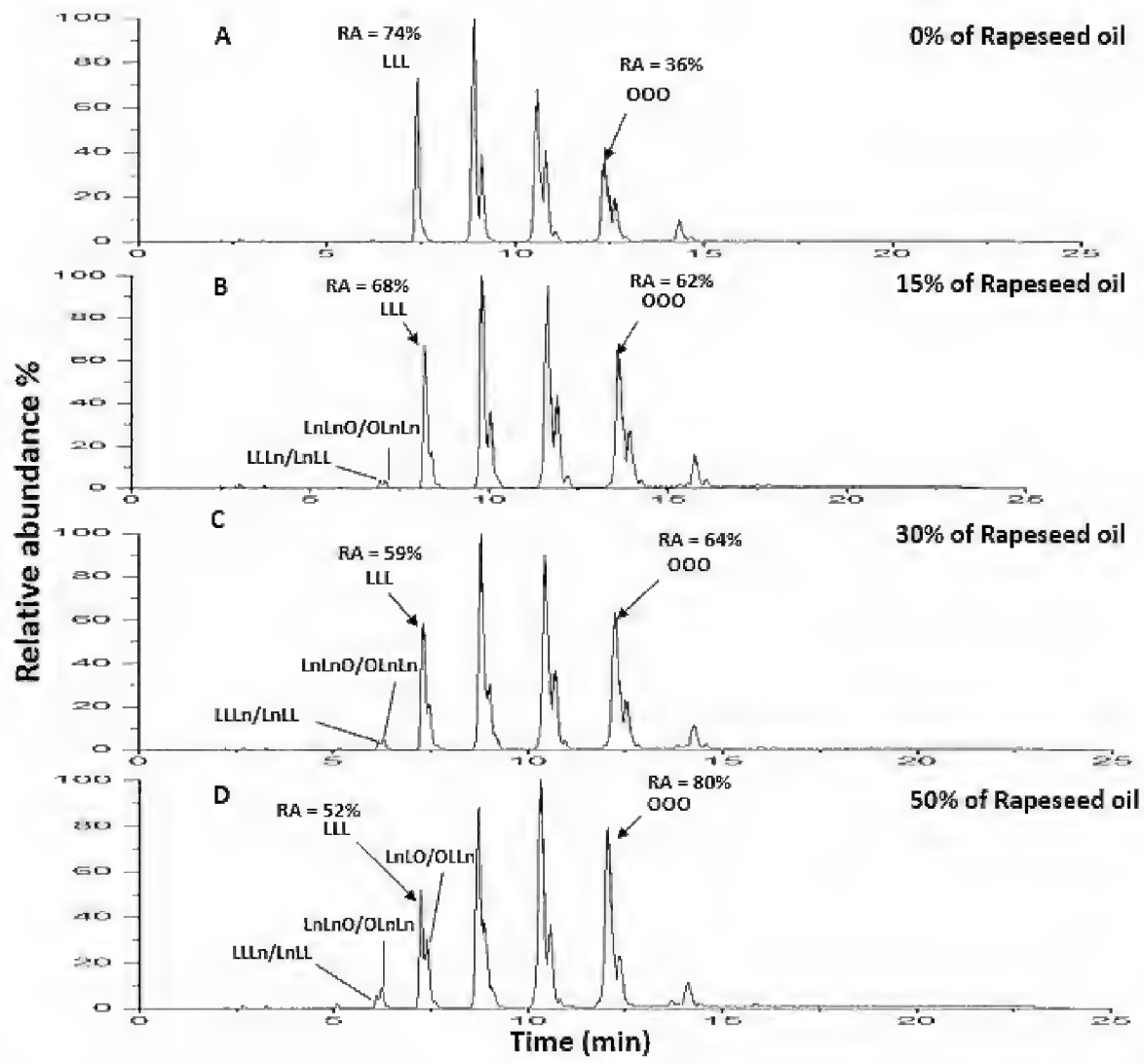

Figure 8. UHPLC-MS chromatograms of **A.** Pure sesame oil; **B.** Sesame oil mixed with 15% of rapeseed oil; **C.** Sesame oil mixed with 30% of rapeseed oil, and **D.** Sesame oil mixed with 50% of rapeseed oil, showing the TAG marker of LLL and OOO. These samples were mixed in the laboratory.

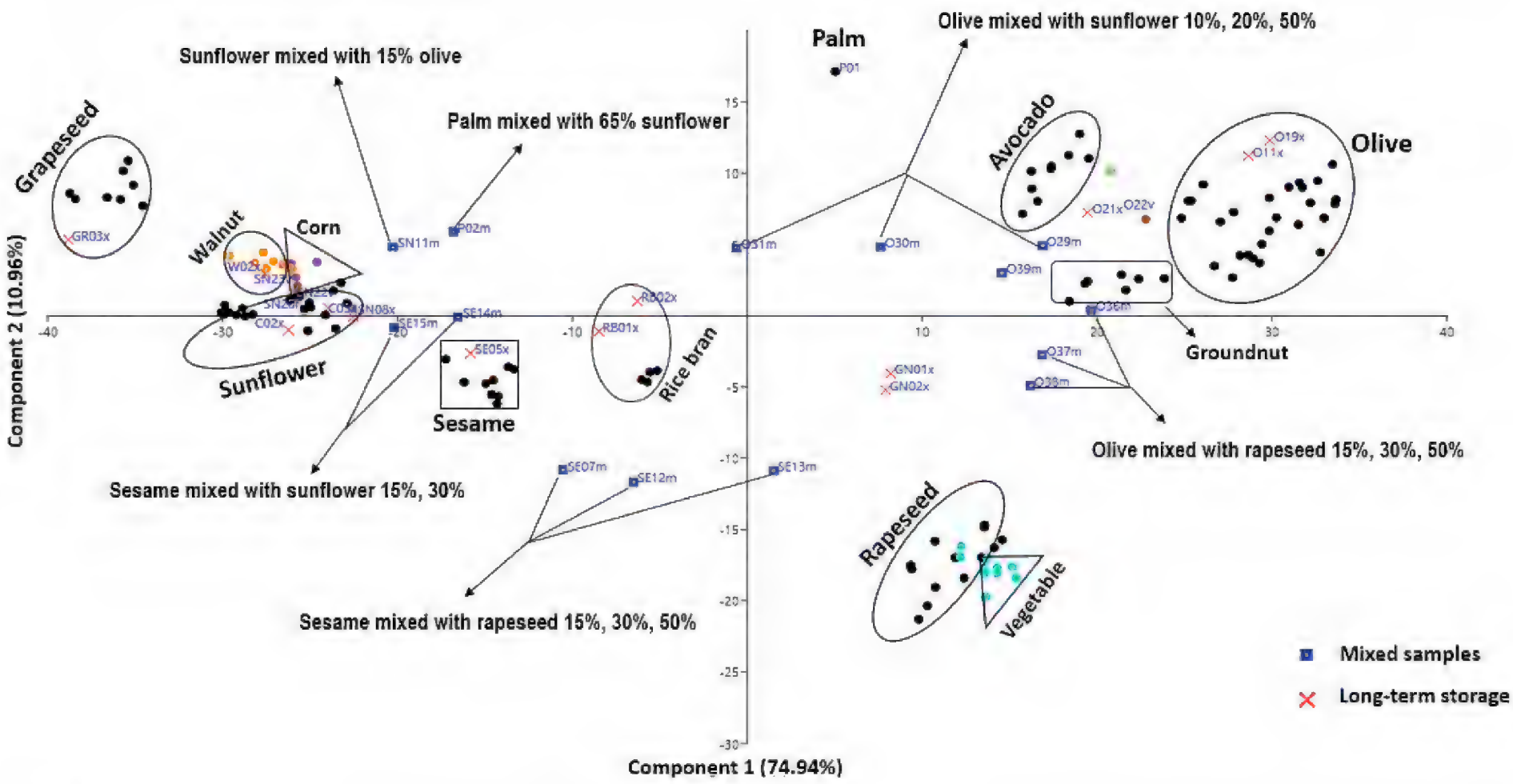

Figure 9. PCA scores plot (PC1 vs PC2) of 12 different kinds of oils, including olive oil, sunflower oil, sesame oil, rapeseed oil, vegetable oil, corn oil, grapeseed oil, walnut oil, rice bran oil, avocado oil and groundnut oil. Adulterated samples (m), deep-fried samples (f), microwave samples (v), and long-term storage samples (x) were included.

account for 74.94% and 10.96% of the total variability, respectively. In published studies, as the concentration of a seed oil in olive oil increases, the PCA score for the mixture moves progressively closer to the pure seed oil's cluster (Ray et al. 2021). In this study, it was demonstrated that olive oil samples (O29m, O30m, and O31m) with increasing concentrations of sunflower oil (10%, 20%, and 50%), respectively, were separated from the olive oil cluster and moved closer to the sunflower oil group gradually as shown in Fig. 9. Similarly, olive oil components (O36m, O37m, and O38m) were distinctly isolated from the olive group and exhibited a gradual shift towards the rapeseed cluster when mixed with 15%, 30%, and 50% of rapeseed oil. Interestingly, the mixed olive component (O39m), which was a blend of 15% sunflower oil and 15% rapeseed oil, located between the olive components mixed with sunflower (between O29m, 10% and O30m, 20% samples) and the components mixed with rapeseed (in line with O36m, 15%), confirming the robustness of the approach. Based on the distributions and loadings plot (Fig. 12), certain TAGs (LLL, LLO*, LnOO*, OOL*, OOO, and OOP*) were important in discriminating the mixtures of olive oil from the pure oils. The combination of OOO, LLL, and LLO* led the olive components mixed with sunflower to be separated clearly from the olive cluster, whereas LnOO*, OOL*, OOO, and OOP* caused the olive oils mixed with rapeseed to be isolated apart from such group on PCA.

Pure sesame oil is compositionally similar to several common seed oils (rich in oleic and linoleic acids), which historically makes the detection of its adulteration challenging (Rizki et al. 2016). Nevertheless, PCA results in this research show that sesame oil samples adulterated with rapeseed or sunflower oil can be distinctly separated from pure sesame oil, plotting between the sesame cluster and the cluster of the adulterant. Specifically, sesame oil components (SE07m, SE12m, SE13m) exhibited a distinct separation from the sesame oil group, gradually moving towards the rapeseed area when blending with 15%, 30%, and 50% of rapeseed oil. Likewise, sesame oil components (SE14m and SE15) were detached from the sesame group and moved progressively towards the sunflower area when blending them with 15% and 30% of sunflower oil, as shown in PCA plot (Fig. 9). Notably, the loadings plot for PC1 (OOO, LLL, and LLO*) and the negative loadings plot for PC2 (LnLO* and OOL*) (Fig. 12) reveal that a combination of OOL*, LLL, LLO*, OOO and LnLO* caused the mixtures of sesame and rapeseed oils to be isolated from the pure cluster, while LLP* and OOL* contributed significantly to the distinction of sesame oils mixed with sunflower from the pure oils on PCA. Additionally, the two commercial blend oil samples (sunflower mixed with olive and palm with sunflower) were readily recognised in PCA. Specifically, the sunflower oil sample (SN11m) mixed with 15% olive oil was far away from the sunflower cluster and located between the sunflower and olive groups, whereas the palm oil (P02m) blended with 65% sunflower was moved closer to the sunflower cluster (Fig. 9). In one example, a 70% canola: 20% grapeseed: 10% olive oil blend was observed to project closest to the pure canola oil cluster, with slight shifts in the PCA coordinates toward

the grapeseed and olive clusters, reflecting its mixed makeup (Ray et al. 2021).

Generally, the results of the cluster analysis (Fig. 13) indicate that there was a significant difference between the blended samples and the pure oils clusters. The mixtures of olive and sesame oils were identified with different distances among the various pure oils, indicating they were successfully recognised. Consequently, the combined use of PCA and cluster analysis effectively succeeded in distinguishing adulterated olive oils, sesame oils, sunflower oil and palm oil from pure samples. There has been limited literature concerning the detection of oil adulteration through TAGs analysis with HPLC-MS (Tsimidou et al. 1987; Lisa et al. 2009; Fasciotti et al. 2010). Importantly, none of the HPLC-MS methods showed a suitable approach for the detection of adulteration in high-value oils having similar TAG profiles (such as sesame blended with sunflower or olive with rapeseed). It should be noted that very few prior studies have demonstrated the detection of adulteration in high-value oils with similar TAG profiles. For example, neither Lísa et al. (2009) nor Fasciotti et al. (2010) specifically addressed blends such as sesame + sunflower or olive + rapeseed, and the analyses in those studies required more than 110 minutes and ~40 minutes, respectively. In contrast, our UHPLC-MS approach achieved separation for these challenging adulterations in only 20 min, and to the best of our knowledge, such discrimination by TAG profiling has not been reported previously.

Degradation of oils under long-term storage

Several oil samples that had been stored in sealed glass bottles for approximately five years in the laboratory at room temperature were re-analysed using the method to assess their stabilities during long-term storage. In general, long-term storage resulted in slight alterations to the overall content of unsaturated TAGs in certain oils. Analysis revealed that the two oils with the highest levels of polyunsaturated TAGs (sunflower and corn) exhibited a decrease in the unsaturated quantity of TAGs, such as LLL and OOO (Table 5). For example, when sunflower sample was analysed immediately after purchase, the relative peak area of LLL was 100%. After five years storage, the relative abundance of LLL in sunflower oil was reduced to 80% (the peak area of LLL has been measured against the base peak), as shown in Fig. 10, suggesting that they may have been subject to autoxidation. By contrast, olive and sesame samples (rich in monounsaturated FAs) were relatively not affected during long-term storage, as shown in Table 5.

During long-term storage the loss of polyunsaturated fatty acids, such as linoleic acid, was found, with the formation of oxidation products being associated with changes in the flavour and nutrient value of oils (Li et al. 2013; Vrbiková et al. 2014; Wroniak et al. 2015; Liu et al. 2019; Alvarruiz et al. 2020). The significant susceptibility of fatty acids to autoxidation was thought to directly correlate with their degree of unsaturation (greater unsaturation tends to lead to higher susceptibility to oxidation) (Gladine et al. 2007; Chávez-Servín et al. 2009; Li et al. 2013). Specifically, polyunsaturated FAs are more prone to oxidation compared to monounsaturated ones. Results in the present study were consistent with earlier research findings, confirming that polyunsaturated oils during long-term storage were subjected to degradation. However, to the best of this author's knowledge, the present study represents the first evaluation of the stability of edible oils during long-term storage based on their TAG profiles.

In order to assess if the oil treatments impact the groupings of the oils, PCA and cluster analysis were utilised. It was illustrated in PCA (Fig. 9) that some oil samples after long-term storage (e.g. GNx, RBx, GR02x, SN08x, and Cx) were shifted from their original clusters, which indicates that changes in their TAG profiles caused shifts in their positions with respect to the fresh oils. Based on the positive and negative loadings from PC1 (OOO, LLL, and LLO*) and the negative lodging from PC3 (OOL*) (Fig. 12), these compounds were the most critical descriptors to the shift of such components from their groups. In contrast, the components of olive oil

Table 5. Mean relative abundances of major TAGs from fresh and long-term storage samples of olive oil, sesame oil, sunflower oil, and corn oil. The individual TAG peak areas of all major identified compounds for these oils are expressed relative to the total peak area.

TAGs	mean TAG abundances % in olive oil		mean TAG abundances % in sesame oil		mean TAG abundances % in sunflower oil		mean TAG abundances % in corn oil	
	Fresh	Long storage	Fresh	Long storage	Fresh	Long storage	Fresh	Long storage
LLL	0.10	-	17.56	18.40	28.00	23.72	26.00	20.00
LLO*	1.74	1.40	24.00	23.13	27.00	30.00	20.00	25.00
LLP*	0.91	1.50	8.82	7.78	9.26	6.80	13.94	14.85
OOL*	13.16	10.56	22.00	19.50	15.44	14.47	13.61	15.35
PLO*	5.81	5.77	7.70	7.13	4.20	2.55	9.90	10.42
OOO	47.00	44.00	14.23	14.43	13.76	13.22	7.48	7.28
OOP*	21.48	24.00	5.16	4.65	2.97	3.00	5.12	4.53
OOS*	5.27	7.55	2.50	3.20	1.72	1.92	0.94	1.17
Overall status	Not affected		Not affected		Slightly affected		Slightly affected	

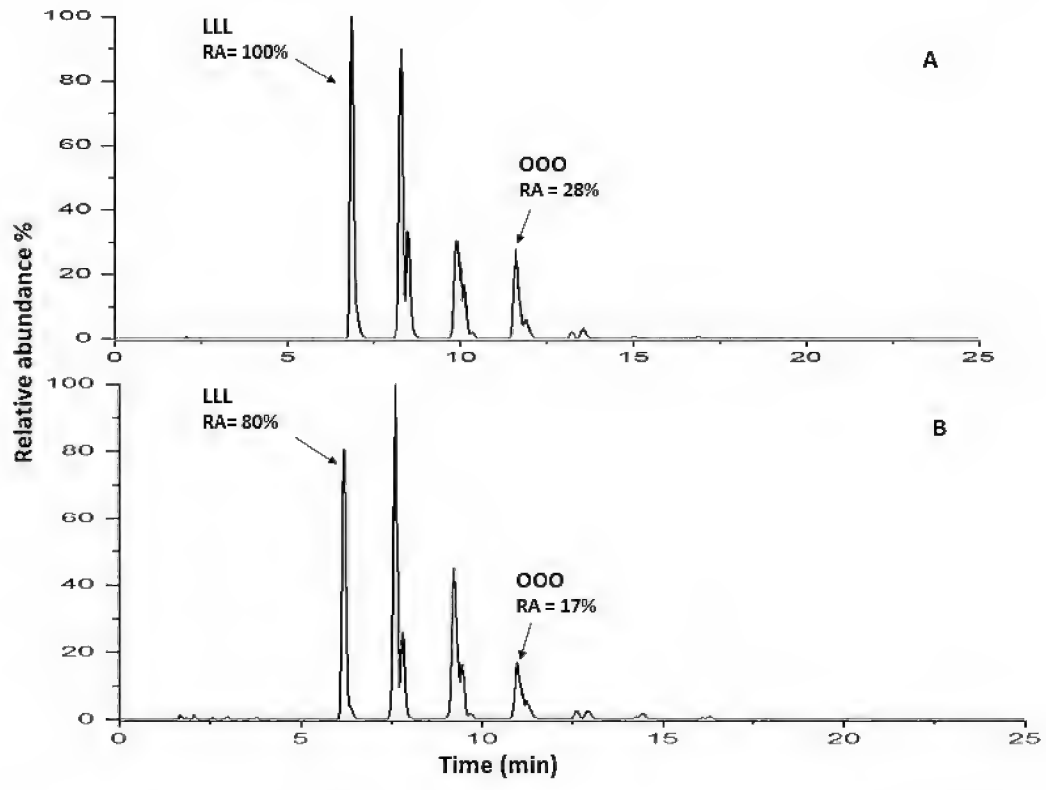

Figure 10. UHPLC-APCI MS chromatograms of **A.** Sunflower oil analysed immediately after purchase; **B.** Sunflower oil analysed after five years.

and sesame oil (O11x, O19x, and SE05x) were not separated from their clusters (Fig. 11), showing that those samples were more stable during long-term storage. This stability might be due to their antioxidant characteristics. Both olive oil and sesame oil have a higher content of antioxidants and monounsaturated FAs (Rohman et al. 2020; Chau et al. 2021). Specifically, these oils contain compounds, such as polyphenols in olive and sesamin in sesame, that contribute to their oxidative stability, which can help protect cells from oxidative stress, as discussed in a review by Rohman et al. (2020). Earlier studies (Ramos et al. 2009; Li et al. 2013) have also reported that oil oxidation was related to the level of unsaturation in fatty acids and the presence of natural antioxidants, in the sense that oils with higher levels of unsaturation and lower antioxidants are more susceptible to oxidation, such as corn oil (rich in polyunsaturated FAs with low antioxidants) versus olive oil (having a high level of monounsaturated FAs and antioxidants).

Cluster analysis produced a dendrogram showing that the distance between Wx, GRx, Cx, SNx, and RBx samples and the fresh oils cluster was larger compared with other oils following long-term storage (Fig. 13). This suggests that these polyunsaturated oils were more susceptible to oxidation than monounsaturated oils, such as olive oil (Ox), most likely due to them containing higher proportions of polyunsaturated TAGs (e.g. LLLn* and LLL) (Li et al. 2013).

Degradation of oils under different heating conditions

The present experiment focused on evaluating olive oil (EVOO) and sunflower oil samples under different

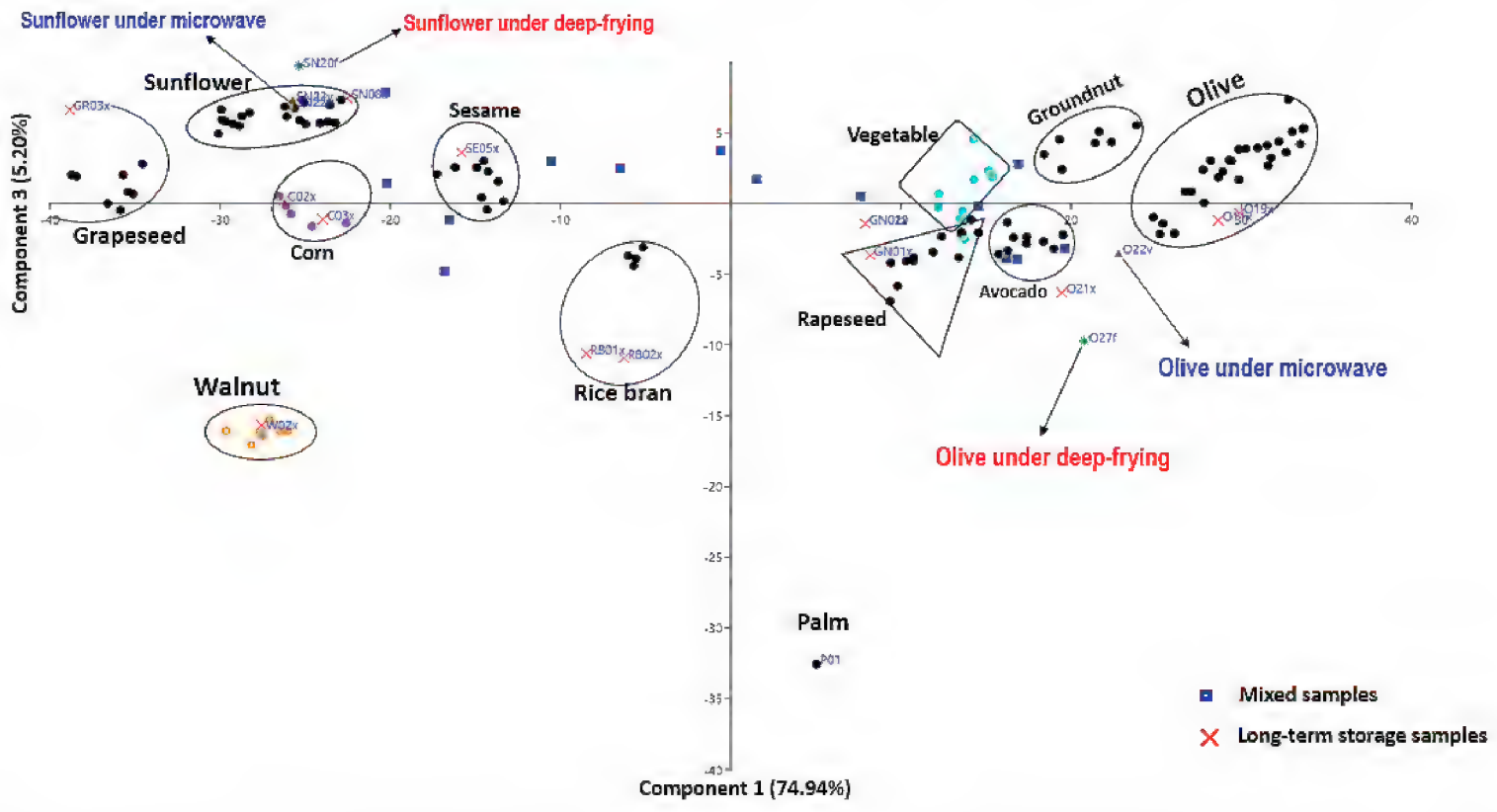

Figure 11. PCA scores plot (PC1 vs PC2) of 12 different kinds of oils, including olive, sunflower, sesame, rapeseed, vegetable, corn, grapeseed, walnut, rice bran, avocado, and groundnut. Adulterated samples (m), deep-fried samples (f), microwave samples (v), and long-term storage samples (x) were included.

heating conditions since these oils are among the most popular and widely consumed edible oils globally. Two different heated oils (sunflower and EVOO) were analysed to determine the TAG distributions after different heating methods were applied to the different oils. Specifically, olive and sunflower samples were heated in an open pan on a hob for about 10 minutes to around 200 °C (regular deep-frying temperature). In addition, the same components were subjected to microwave heating for 5 minutes to gain insights into which method (pan-heated and microwave) is more or less prone to causing oil degradation.

The TAG distributions of fresh and heated extra virgin (EVOO) olive and pure sunflower oils were analysed by the PCA and cluster analysis to estimate the extent of variation occurring with two different cooking conditions (pan-heated and microwave). The PCA scores plot shown in Fig. 11 shows that the microwave olive sample (O22v) and the deep-fried sample (O27f) were significantly separated from the fresh olive cluster. Notably, the olive sample (O21x), which was obtained from Saudi Arabia as a fresh sample but was stored in a domestic kitchen where it had been exposed to sunlight and a temperature of about 40 °C for a relatively long period, was obviously close to the O22v and O27f samples (Fig. 11), suggesting that it may also have been subject to autoxidation. There was no clear distinction between the microwave sunflower component (SN22v) and the fresh sunflower group, indicating that microwave cooking has not caused a major change in the TAGs abundances for sunflower oil. By contrast, the deep-fried sunflower (SN20f) was clearly separated from the fresh oils cluster, as shown in Fig. 11. The loadings plot for PC1 vs. PC3 (Fig. 12) reveals that a combination of LLL, LLO*, OOL*, OOO, and OOP* caused the heated oils (O22v, O27f, and SN20f) to be clearly distinguished from fresh oils on PCA.

The dendrogram shows the same distance between O21x and O22v, with O27f being the next nearest composition (Fig. 13). This result indicates that the deep-fried olive sample was affected more than the microwave olive sample. Notably, the results of the cluster analysis (Fig. 13) indicate that there was no significant difference between the cooked samples (SN22v and SN20f) and the fresh sunflower cluster. In contrast, the olive oil samples (O22v and O27f) were separated from the fresh olive oils and clustered with the oil (O21x) exposed to sunlight and heat. This further supports the finding from the PCA that the cooked sunflower samples were not significantly different from the fresh samples. Overall, sunflower samples under cooking conditions were likely more stable than olive samples. This might be attributed to the fact that oil stability during heating is highly correlated with their respective smoke points. The smoking point of an oil is the temperature at which it begins to produce visible smoke continuously, emitting unpleasant odour and flavour (Katragadda et al. 2010). Hence, oils with higher smoke points are generally more stable (Alvarenga et al. 2018). According to the literature (Fullana et al. 2004; Katragadda et al. 2010; Alvarenga et al. 2018), sunflower oil has a higher smoke point (230 °C) than unrefined olive oil, such as EVOO (195 °C), suggesting that sunflower oil could be a better choice for frying.

In general, these results support the conclusions derived from the earlier PCA findings and further contribute to successfully distinguishing the degraded oils from fresh samples.

Conclusion

This study presents a rapid UHPLC-MS TAG-profiling method, combined with chemometric analysis, for authenticating edible oils and detecting oil adulteration and degradation. The method successfully distinguished twelve different edible oils, with PCA and HCA revealing clear clustering by oil type. Notably, the generic "vegetable oil" sample was correctly identified as rapeseed oil based on TAG profile, highlighting the approach's ability to verify labelling. The method also identified the adulteration of high-value olive and sesame oils with cheaper oils, even when the adulterant had a similar TAG profile, a feat not previously achieved by HPLC-MS methods in such a short analysis time. In addition, the approach detected compositional changes in oils after long-term storage, particularly in polyunsaturated oils (sunflower, corn, etc.), while confirming the relative stability of monounsaturated, antioxidant-rich oils (olive, sesame) over time.

It should be noted that this UHPLC-APCI-MS method does not distinguish TAG positional isomers (*sn*-1 vs *sn*-3), meaning the exact fatty acid arrangement on the glycerol backbone is not determined. However, this did not impede the discrimination of oils at the genus level. Another limitation is the relatively small sample size for certain oils (e.g., only one palm oil sample), which may limit statistical robustness for those cases; expanding the sample set in future studies would strengthen the conclusions. Additionally, the adulteration experiments in this study used adulterant levels down to 10%—lower concentrations of adulteration were not tested and would be a subject for future investigation to establish the method's detection threshold.

Despite these limitations, the proposed method proved to be a rapid and effective approach for authenticating edible oil. With its minimal sample preparation and short run time, it could be utilised by industry and regulatory agencies as a routine screening tool to detect oil adulteration or degradation, issues that carry significant economic and health implications. Moreover, a key finding to emerge from this study is that this type of simple and reliable identification tool does not require specialised, trained chemists. Thus, it has great potential for universal adoption.

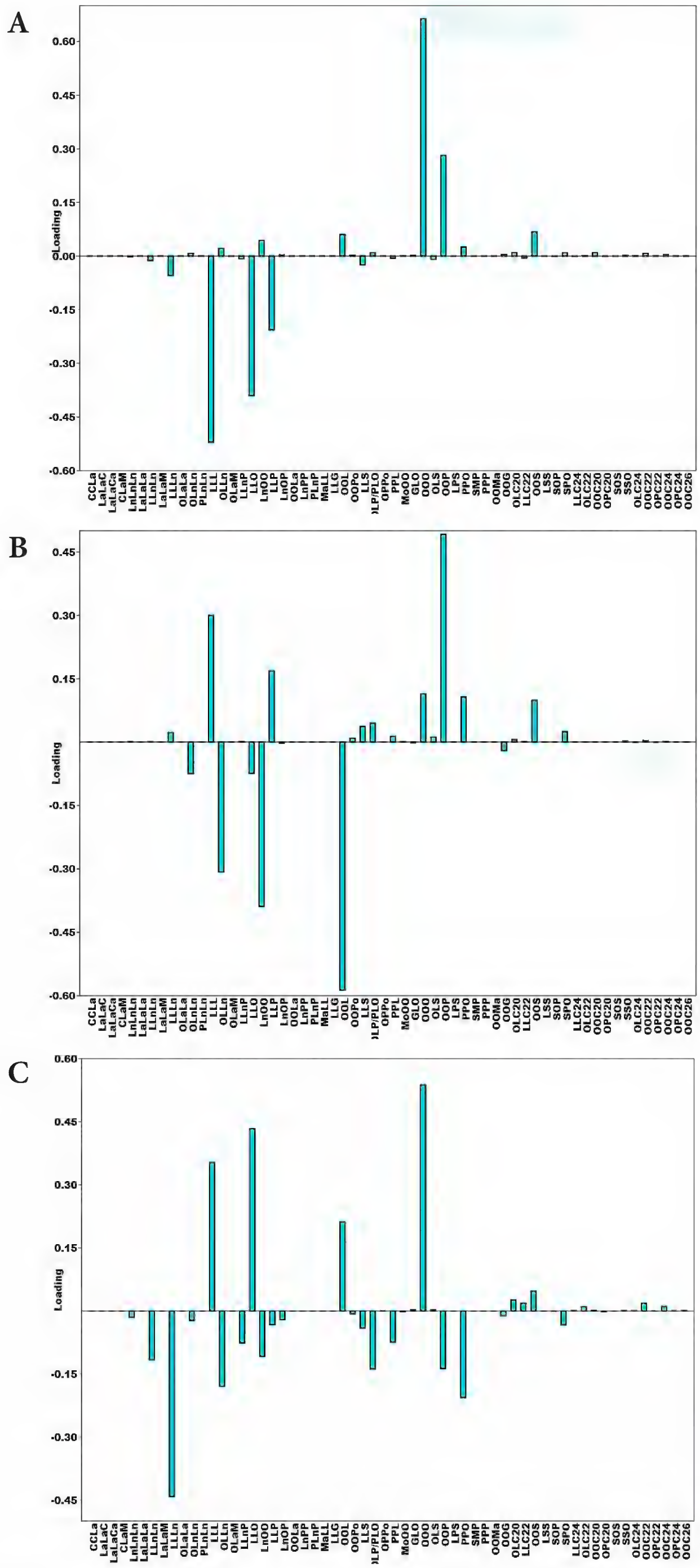
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Figure 12. A. PC1 loadings plot for Fig. 9; **B.** PC2 loadings plot for Fig. 9; **C.** PC3 loadings plot for Fig. 11.

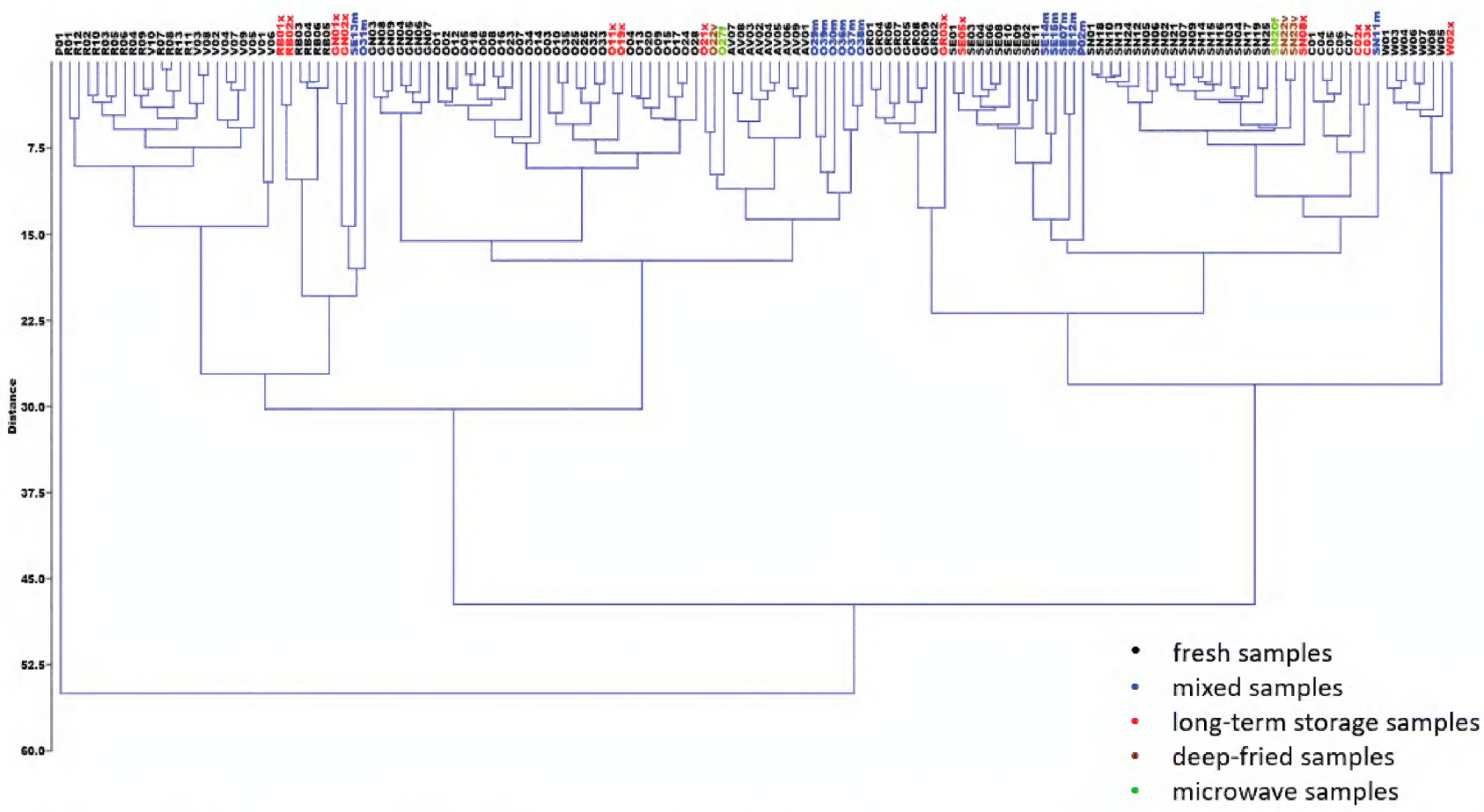

Figure 13. Hierarchical cluster analysis by linkage and distance obtained from 12 different kinds of oils including olive oil (O), sunflower oil (SN), sesame oil (SE), rapeseed oil (R), vegetable oil (V), corn oil (C), grapeseed oil (GR), walnut oil (W), rice bran oil (RB), avocado oil (AV), palm oil (P), and groundnut oil (GN). Adulterated samples (m), deep-fried samples (f), microwave samples (v), and long-term storage samples (x) were included.

Author contribution

Faris Alfifi played a major role in preparing the methodology, bringing resources, writing the original draft and preparing figures and tables. Brendan Keely played a major role in supervising the work and reviewing the whole final manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Data collection and analysis

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

Alvarenga BR, Xavier FA, Soares FL, Carneiro RL (2018) Thermal stability assessment of vegetable oils by Raman spectroscopy and chemometrics. Food Analytical Methods 11(7): 1969–1976. https://doi.org/10.1007/s12161-018-1160-y

Alvarruiz A, Pardo JE, Copete ME, Miguel C de, Rabadán A, López E, Álvarez-Ortí M (2020) Evolution of virgin olive oil during long-term storage. Journal of Oleo Science 69(8): 809–814. https://doi.org/10.5650/jos.ess19258

Barison A, Pereira da Silva CW, Campos FR, Simonelli F, Lenz CA, Ferreira AG (2010) A simple methodology for the determination of fatty acid composition in edible oils through 1H NMR spectroscopy. Magnetic Resonance in Chemistry 48: 642–650. https://doi.org/10.1002/mrc.2629

Bastos LC, de Almeida Costa EA, Pereira PA (2017) Development, validation and application of an UFLC-Dad-ESI-MS method for determination of carbonyl compounds in soybean oil during continuous heating. Food Chemistry 218: 518–524. https://doi.org/10.1016/j.foodchem.2016.09.036

Ben Arfa K, de Person M, Hmida D, Bleton J, Boukhchina S, Tchapla A, Héron S, Moussa F (2017) UHPLC-APCI-MS profiling of triacylglycerols in vegetable oils-application to the analysis of four North African sesame seed varieties. Food Analytical Methods 10(8): 2827–2838. https://doi.org/10.1007/s12161-017-0851-0

Bosque-Sendra JM, Cuadros-Rodríguez L, Ruiz-Samblás C, de la Mata AP (2012) Combining chromatography and chemometrics for the characterisation and authentication of fats and oils from triacylglycerol compositional data-a review. Analytica Chimica Acta 724: 1–11. https://doi.org/10.1016/j.aca.2012.02.041

Bouabdallah I, Bouali I, Martinez-Force E, Albouchi A, Perez Camino MC, Boukhchina S (2014) Composition of fatty acids, triacylglycerols and polar compounds of different walnut varieties (Juglans regia L.) from Tunisia. Natural Product Research 28(21): 1826–1833. https://doi.org/10.1080/14786419.2014.950573

Byrdwell W (2005) Atmospheric pressure ionisation techniques in modern lipid analysis. Modern Methods for Lipid Analysis by Liquid Chromatography.

Cao G, Ding C, Ruan D, Chen Z, Wu H, Hong Y, Cai Z (2019) Gas chromatography-mass spectrometry-based profiling reveals six monoglycerides as markers of used cooking oil. Food Control 96: 494–498. https://doi.org/10.1016/j.foodcont.2018.10.013

Cao G, Hong Y, Wu H, Chen Z, Lu M, Cai Z (2021) Visual authentication of edible vegetable oil and used cooking oil using MALDI Imaging Mass Spectrometry. Food Control 125: 107966. https://doi.org/10.1016/j.foodcont.2021.107966

Chanidou M (2020) Development of a UHPLC/MS method for the analysis of triacylglycerols in oils, fats and milk products. PhD thesis, University of York, UK.

Chau C-F, Ciou J-Y, Wu C-L (2021) Commercialised sesame oil analysis: quality characterisation and oxidative stability of blended sesame oil. ACS Food Science & Technology 1(7): 1222–1227. https://doi.org/10.1021/acsfoodscitech.1c00008

Chávez-Servín JL, Castellote AI, Martín M, Chifré R, Carmen López-Sabater M (2009) Stability during storage of LC-PUFA-supplemented infant formula containing single cell oil or egg yolk. Food Chemistry 113(2): 484–492. https://doi.org/10.1016/j.foodchem.2008.07.082

Che Zain MS, Osman MF, Lee SY, Shaari K (2021) UHPLC-UV/PDA method validation for simultaneous quantification of luteolin and apigenin derivatives from Elaeis guineensis leaf extracts: An application for antioxidant herbal preparation. Molecules 26(4): 1084. https://doi.org/10.3390/molecules26041084

Choe E, Min DB (2007) Chemistry of deep-fat frying oils. Journal of Food Science 72(5): R77–R86. https://doi.org/10.1111/j.1750-3841.2007.00352.x

Choudhary A, Gupta N, Hameed F, Choton S (2020) An overview of food adulteration: Concept, sources, impact, challenges and detection. International Journal of Chemical Studies 8(1): 2564–2573. https://doi.org/10.22271/chemi.2020.v8.i1am.8655

Endo Y, Ohta A, Kido H, Kuriyama M, Sakaguchi Y, Takebayashi S, Hirai H, Murakami C, Wada S (2011) Determination of triacylglycerol composition in vegetable oils using high-performance liquid chromatography: A collaborative study. Journal of Oleo Science 60(9): 451–456. https://doi.org/10.5650/jos.60.451

European Medicines Agency (2022) Ich Q2(R2) validation of Analytical Procedures - scientific guideline European Medicines Agency. https://www.ema.europa.eu/en/ich-q2r2-validation-analytical-procedures-scientific-guideline

Fasciotti M, Pereira Netto AD (2010) Optimization and application of methods of triacylglycerol evaluation for characterization of olive oil adulteration by soybean oil with HPLC-APCI-MS-MS. Talanta 81(3): 1116–1125. https://doi.org/10.1016/j.talanta.2010.02.006

Fauconnot L, Hau J, Aeschlimann J-M, Fay L-B, Dionisi F (2003) Quantitative analysis of triacylglycerol regioisomers in fats and oils using reversed-phase high-performance liquid chromatography and atmospheric pressure chemical ionization mass spectrometry. Rapid Communications in Mass Spectrometry 18(2): 218–224. https://doi.org/10.1002/rcm.1317

Food and Agriculture Organization of the United Nations (1998) Validation of analytical methods for food control: Report of a joint FAO/IAEA expert consultation, Vienna, Austria, 2–4 December 1997. Rome. https://www.fao.org/3/w8420e/w8420e.pdf

Fragaki G, Spyros A, Siragakis G, Salivaras E, Dais P (2005) Detection of extra virgin olive oil adulteration with Lampante olive oil and refined olive oil using nuclear magnetic resonance spectroscopy and multivariate statistical analysis. Journal of Agricultural and Food Chemistry 53(8): 2810–2816. https://doi.org/10.1021/jf040279t

Fullana A, Carbonell-Barrachina Angel A, Sidhu S (2004) Volatile aldehyde emissions from heated cooking oils. Journal of the Science of Food and Agriculture 84(15): 2015–2021. https://doi.org/10.1002/jsfa.1904

Gladine C, Morand C, Rock E, Gruffat D, Bauchart D, Durand D (2007) The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. Animal Feed Science and Technology 139(3–4): 257–272. https://doi.org/10.1016/j.anifeedsci.2007.01.015

Granato D, Santos JS, Escher GB, Ferreira BL, Maggio RM (2018) Use of principal component analysis (PCA) and hierarchical cluster Analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. Trends in Food Science & Technology 72: 83–90. https://doi.org/10.1016/j.tifs.2017.12.006

Green HS, Li X, De Pra M, Lovejoy KS, Steiner F, Acworth IN, Wang SC (2020) A rapid method for the detection of extra virgin olive oil adulteration using UHPLC-CAD profiling of triacylglycerols and PCA. Food Control 107: 106773. https://doi.org/10.1016/j.foodcont.2019.106773

Grob K (1981) Degradation of triglycerides in gas chromatographic capillaries: Studies by reversing the column. Journal of Chromatography A 205(2): 289–296. https://doi.org/10.1016/S0021-9673(00)82657-6

Holčapek M, Jandera P, Zderadička P, Hrubá L (2003) Characterization of triacylglycerol and diacylglycerol composition of plant oils using high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A 1010(2): 195–215. https://doi.org/10.1016/S0021-9673(03)01030-6

Holčapek M, Lísa M, Jandera P, Kabátová N (2005) Quantitation of triacylglycerols in plant oils using HPLC with APCI-MS, evaporative light-scattering, and UV detection. Journal of Separation Science 28(12): 1315–1333. https://doi.org/10.1002/jssc.200500088

Huq AKO, Uddin I, Ahmed E, Siddique MAB, Zaher MA, Nigar S (2022) Fats and oils adulteration: Present scenario and rapid detection techniques. Food Research 6(1): 5–11. https://doi.org/10.26656/fr.2017.6(1).116

Ikawa M, Sasner JJ, Haney JF (1997) Inhibition of Chlorella growth by degradation and related products of linoleic and linolenic acids and the possible significance of polyunsaturated fatty acids in phytoplankton ecology. Hydrobiologia 356(1/3): 143–148. https://doi.org/10.1023/A:1003103726520

Jakab A, Héberger K, Forgács E (2002) Comparative analysis of different plant oils by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A 976(1–2): 255–263. https://doi.org/10.1016/S0021-9673(02)01233-5

Jehangir M, Ahmed M, Imtiaz Shafiq M, Samad A, Iftikhar-ul-Haq (2017) UHPLC-PDA assay for simultaneous determination of vitamin D3and menaquinone-7 in pharmaceutical solid dosage formulation. Journal of Analytical Methods in Chemistry 2017: 1–9. https://doi.org/10.1155/2017/1208753

Kalogiouri NP, Manousi N, Mourtzinos I, Rosenberg E, Zachariadis GA (2021) A Rapid GC-FID method for the determination of fatty acids in walnut oils and their use as markers in authenticity studies. Food Analytical Methods 15(3): 761–771. https://doi.org/10.1007/s12161-021-02157-3

Kamm W, Dionisi F, Hischenhuber C, Engel K-H (2001) Authenticity assessment of fats and oils. Food Reviews International 17(3): 249–290. https://doi.org/10.1081/FRI-100104702

Katragadda HR, Fullana A, Sidhu S, Carbonell-Barrachina ÁA (2010) Emissions of volatile aldehydes from heated cooking oils. Food Chemistry 120(1): 59–65. https://doi.org/10.1016/j.foodchem.2009.09.070

Kou Y, Li Q, Liu X, Zhang R, Yu X (2018) Efficient detection of edible oils adulterated with used frying oils through pe-film-based FTIR spectroscopy combined with DA and PLS. Journal of Oleo Science 67(9): 1083–1089. https://doi.org/10.5650/jos.ess18029

La Nasa J, Ghelardi E, Degano I, Modugno F, Colombini MP (2013) Core shell stationary phases for a novel separation of triglycerides in plant oils by high performance liquid chromatography with electrospray-quadrupole-time of Flight Mass Spectrometer. Journal of Chromatography A 1308: 114–124. https://doi.org/10.1016/j.chroma.2013.08.015

Lerma-García MJ, Lusardi R, Chiavaro E, Cerretani L, Bendini A, Ramis-Ramos G, Simó-Alfonso EF (2011) Use of triacylglycerol profiles established by high performance liquid chromatography with ultraviolet-visible detection to predict the botanical origin of vegetable oils. Journal of Chromatography A 1218(42): 7521–7527. https://doi.org/10.1016/j.chroma.2011.07.078

Li H, Fan Y, Li J, Tang L, Hu J, Deng Z (2013) Evaluating and predicting the oxidative stability of vegetable oils with different fatty acid compositions. Journal of Food Science 78(4): H633–H641. https://doi.org/10.1111/1750-3841.12089

Lichtenstein AH (2013) Encyclopedia of Human Nutrition, 3rd edn.

Lísa M, Holčapek M, Boháč M (2009) Statistical evaluation of triacylglycerol composition in plant oils based on high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry data. Journal of Agricultural and Food Chemistry 57(15): 6888–6898. https://doi.org/10.1021/jf901189u

Liu K, Liu Y, Chen F (2019) Effect of storage temperature on lipid oxidation and changes in nutrient contents in peanuts. Food Science & Nutrition 7(7): 2280–2290. https://doi.org/10.1002/fsn3.1069

Lucci P, Moret S, Buchini F, Ferlat G, Conte L (2018) Improved analysis of olive oils triacylglycerols by UHPLC-charged aerosol detection. Journal of Food Composition and Analysis 66: 230–236. https://doi.org/10.1016/j.jfca.2017.12.028

Makarov A, Scigelova M (2010) Coupling liquid chromatography to orbitrap mass spectrometry. Journal of Chromatography A 1217(25): 3938–3945. https://doi.org/10.1016/j.chroma.2010.02.022

Marikkar JMN, Ghazali HM, Che Man YB, Peiris TSG, Lai OM (2005) Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis. Food Chemistry 91(1): 5–14. https://doi.org/10.1016/j.foodchem.2004.01.080

Mottram HR, Woodbury SE, Evershed RP (1997) Identification of triacylglycerol positional isomers present in vegetable oils by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. Rapid Communications in Mass Spectrometry 11(12): 1240–1252. https://doi.org/10.1002/(SICI)1097-0231(199708)11:12<1240::AID-RCM990>3.0.CO;2-5

Peršurić Ž, Saftić L, Mašek T, Kraljević Pavelić S (2018) Comparison of triacylglycerol analysis by MALDI-TOF/MS, fatty acid analysis by GC-MS and non-selective analysis by NIRS in combination with chemometrics for determination of extra virgin olive oil geographical origin. A case study. LWT 95: 326–332. https://doi.org/10.1016/j.lwt.2018.04.072

Qian Y, Rudzińska M, Grygier A, Przybylski R (2020) Determination of triacylglycerols by HTGC-FID as a sensitive tool for the identification of rapeseed and olive oil adulteration. Molecules 25(17): 3881. https://doi.org/10.3390/molecules25173881

Ramos MJ, Fernández CM, Casas A, Rodríguez L, Pérez Á (2009) Influence of fatty acid composition of raw materials on biodiesel properties. Bioresource Technology 100(1): 261–268. https://doi.org/10.1016/j.biortech.2008.06.039

Ray CL, Gawenis JA, Greenlief CM (2021) A new method for olive oil screening using multivariate analysis of proton NMR spectra. Molecules 27(1): 213. https://doi.org/10.3390/molecules27010213

Řezanka T, Pádrová K, Sigler K (2017) Regioisomeric and enantiomeric analysis of triacylglycerols. Analytical Biochemistry 524: 3–12. https://doi.org/10.1016/j.ab.2016.05.028

Rizki H, Terouzi W, Kzaiber F, Hanine H, Oussama A (2016) Quantification of adulterations in sesame oil with inferior edible oils by using ATR-FTIR coupled to Chemometrics. IOSR Journal of Environmental Science, Toxicology and Food Technology 10(08): 138–145. https://doi.org/10.9790/2402-100802138145

Rohman A, Ghazali MAI, Windarsih A, Irnawati I, Riyanto S, Yusof FM, Mustafa S (2020) Comprehensive review on application of FTIR spectroscopy coupled with chemometrics for authentication analysis of fats and oils in the food products. Molecules 25(22): 5485. https://doi.org/10.3390/molecules25225485

Salerno TM, Oteri M, Arena P, Trovato E, Sciarrone D, Donato P, Mondello L (2023) Fast triacylglycerol fingerprinting in edible oils by subcritical solvent chromatography. Separations 10(1): 56. https://doi.org/10.3390/separations10010056

Salghi R, Armbruster W, Schwack W (2014) Detection of argan oil adulteration with vegetable oils by high-performance liquid chromatography-evaporative light scattering detection. Food Chemistry 153: 387–392. https://doi.org/10.1016/j.foodchem.2013.12.084

Sante (2020) Sante - 2020 annual food fraud report: Fight against food fraud in Europe continued despite the COVID-19 pandemic. [Retrieved January 17: 2023] https://ec.europa.eu/newsroom/sante/items/719585/en

Sutton PA, Rowland SJ (2012) High temperature gas chromatography-time-of-flight-mass spectrometry (HTGC-TOF-MS) for high-boiling compounds. Journal of Chromatography A 1243: 69–80. https://doi.org/10.1016/j.chroma.2012.04.044

Tamba Sompila AWG, Héron S, Hmida D, Tchapla A (2017) Fast non-aqueous reversed-phase liquid chromatography separation of triacylglycerol regioisomers with isocratic mobile phase. application to different oils and fats. Journal of Chromatography B 1041–1042: 151–157. https://doi.org/10.1016/j.jchromb.2016.12.030

Tang F, Green HS, Wang SC, Hatzakis E (2021) Analysis and authentication of avocado oil using high resolution NMR spectroscopy. Molecules 26(2): 310. https://doi.org/10.3390/molecules26020310

Tsimidou M, Macrae R, Wilson I (1987) Authentication of virgin olive oils using principal component analysis of triglyceride and Fatty Acid Profiles: Part 2-detection of adulteration with other vegetable oils. Food Chemistry 25(4): 251–258. https://doi.org/10.1016/0308-8146(87)90011-2

Tu A, Du Z, Qu S (2016) Rapid profiling of triacylglycerols for identifying authenticity of edible oils using supercritical fluid chromatography-quadruple time-of-flight mass spectrometry combined with

chemometric tools. Analytical Methods 8(21): 4226–4238. https://doi.org/10.1039/C6AY00970K

Vaclavik L, Hrbek V, Cajka T, Rohlik B-A, Pipek P, Hajslova J (2011) Authentication of animal fats using Direct Analysis in real time (DART) ionization–mass spectrometry and chemometric tools. Journal of Agricultural and Food Chemistry 59(11): 5919–5926. https://doi.org/10.1021/jf200734x

Vigli G, Philippidis A, Spyros A, Dais P (2003) Classification of edible oils by employing 31P and 1H NMR spectroscopy in combination with multivariate statistical analysis. A proposal for the detection of seed oil adulteration in virgin olive oils. Journal of Agricultural and Food Chemistry 51(19): 5715–5722. https://doi.org/10.1021/jf030100z

Vrbiková L, Schmidt Š, Kreps F, Tmáková L, Čertík M, Sekretár S (2014) Degradation of selected nutrients in sunflower oils during long-term storage. Czech Journal of Food Sciences 32(6): 595–600. https://doi.org/10.17221/176/2014-CJFS

Wabaidur SM, AlAmmari A, Aqel A, AL-Tamrah SA, Alothman ZA, Ahmed AYB (2016) Determination of free fatty acids in olive oils by UPHLC-MS. Journal of Chromatography B 1031: 109–115. https://doi.org/10.1016/j.jchromb.2016.07.040

Wroniak M, Rękas A (2015) Nutritional value of cold-pressed rapeseed oil during long term storage as influenced by the type of packaging material, exposure to light & oxygen and storage temperature. Journal of Food Science and Technology 53(2): 1338–1347. https://doi.org/10.1007/s13197-015-2082-y

Wu Y, Xu F, Ji S, Ji J, Qin F, Ju X, Wang L (2019) Influence of photooxidation on the lipid profile of rapeseed oil using UHPLC-QTOF-MS and Multivariate Data Analysis. Analytical Methods 11(22): 2903–2917. https://doi.org/10.1039/C9AY00385A

Xing C, Yuan X, Wu X, Shao X, Yuan J, Yan W (2019) Chemometric classification and quantification of sesame oil adulterated with other vegetable oils based on fatty acids composition by gas chromatography. LWT 108: 437–445. https://doi.org/10.1016/j.lwt.2019.03.085

Xu S-ling, Wei F, Xie Y, Lv X, Dong X-yan, Chen H (2018) Research advances based on mass spectrometry for profiling of triacylglycerols in oils and fats and their applications. Electrophoresis 39(13): 1558–1568. https://doi.org/10.1002/elps.201700481

Yang T-L, Huang C-L, Lee C-P (2021) Utilizing AgNPt-SALDI to classify edible oils by multivariate statistics of Triacylglycerol Profile. Molecules 26(19): 5880. https://doi.org/10.3390/molecules26195880

Zarringhalami S, Sahari MA, Barzegar M, Hamidi-Esfehani Z (2011) Changes in oil content, chemical properties, fatty acid composition and triacylglycerol species of tea seed oil during maturity period. Journal of Food Biochemistry 35(4): 1161–1169. https://doi.org/10.1111/j.1745-4514.2010.00442.x

Zeb A (2012) Triacylglycerols composition, oxidation and oxidation compounds in camellia oil using liquid chromatography-mass spectrometry. Chemistry and Physics of Lipids 165(5): 608–614. https://doi.org/10.1016/j.chemphyslip.2012.03.004

Zhang Q, Saleh ASM, Chen J, Shen Q (2012) Chemical alterations taken place during deep-fat frying based on certain reaction products: A Review. Chemistry and Physics of Lipids 165(6): 662–681. https://doi.org/10.1016/j.chemphyslip.2012.07.002

Zhao H, Zhan Y, Xu Z, John Nduwamungu J, Zhou Y, Powers R, Xu C (2022) The application of machine-learning and Raman spectroscopy for the rapid detection of edible oils type and adulteration. Food Chemistry 373: 131471. https://doi.org/10.1016/j.foodchem.2021.131471

Zhou S, Xu Z, Liu F (2017) Method for determining the optimal number of clusters based on agglomerative hierarchical clustering. IEEE Transactions on Neural Networks and Learning Systems 28(12): 3007–3017. https://doi.org/10.1109/TNNLS.2016.2608001

Zou M-Q, Zhang X-F, Qi X-H, Ma H-L, Dong Y, Liu C-W, Guo X, Wang H (2009) Rapid authentication of olive oil adulteration by Raman spectrometry. Journal of Agricultural and Food Chemistry 57(14): 6001–6006. https://doi.org/10.1021/jf900217s